Journal of
DAIRY SCIENCE

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on
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September 13-15, 1960

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Manuscripts for publication in the Journal of Dairy Science should be submitted to the editor, E. O. Herreid, at the address above. See January, 19.., issue, page 103, for information concerning the preparation of manuscripts, or send for reprint, "Information for Authors."

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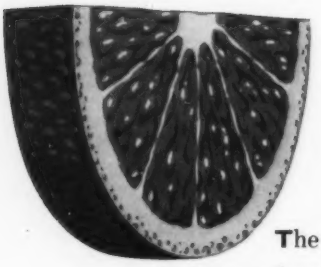

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PEOPLE AND EVENTS

H. F. Judkins Awarded Honorary Degree

H. F. Judkins

Professor H. F. JUDKINS, Secretary-Treasurer of the American Dairy Science Association, was awarded the Honorary Degree of Doctor of Science by the University of New Hampshire at its June Commencement exercises. The citation is reproduced:

HENRY FOREST JUDKINS, graduate of the University in the Class of 1911, you have brought distinction to your profession and have faithfully served your Alma Mater as a member of the faculty, of the Board of Trustees, and of numerous alumni and University committees. Your years as teacher, author, and businessman comprise outstanding service to the dairy industry of the United States. After teaching and conducting research in four state universities, you turned from the classroom to a career in industry, culminating in the Directorship of Production and then the Vice-Presidency of the National Dairy Products Company. Your nominal retirement in 1955 only redirected your service into several offices in the American Dairy Science Association, which you had already served as President. Within the last month the publication of your second textbook has shown the continuing vigor of your professional life, still matched by your characteristic warmth, humor, and wise judgment. The University of New Hampshire is proud to recognize these attainments by conferring upon you, which I now do by the authority vested in me by the Board of Trustees, the degree of Doctor of Science, with all its rights, honors, and privileges.

ELDON L. JOHNSON
June 12, 1960 President

USDA Distinguished Service Award to H. C. Trelogan

H. C. TRELOGAN, Assistant Administrator, Agricultural Marketing Service, Washington, D. C.

For vision and leadership in developing research to solve dynamic and complicated marketing problems during a period of extensive transition in marketing organization and practices.

Dr. Trelogan joined USDA in 1939 as an economist with the Farm Credit Administration. He served the Department in a number of research positions and became Assistant to the Administrator, Research and Marketing Act, in 1947. Currently, Dr. Trelogan is Assistant Administrator, Marketing Research, Agricultural Marketing Service. Before this, he was Director of the Marketing Research Division.

H. C. Trelogan

In addition to his Department duties, Dr. Trelogan has served as president of the American Farm Economic Association, 1957-58, faculty member of the USDA Graduate School since 1948, U. S. delegate to the International Dairy Congress at The Hague in 1953, and in other special posts. In 1952 he received a USDA Superior Service Award.

Dr. Trelogan was born in Versailles, Pa. He received his undergraduate training in agriculture at West Virginia University and his Master's degree in Dairy Husbandry and Doctorate degree in Agricultural Economics from the University of Minnesota.

University of California (Davis) News

J. M. BODA, associate professor of animal husbandry at the University of California, Davis, left in July for New Zealand to spend nine months in research on the bloat problem of cattle in that country.

Supported by a Fulbright research grant, the animal physiologist will work at the Plant Chemistry division in Palmerston North. A. T. JOHNS, director of the division, requested his particiation in the New Zealand bloat studies.

Boda will be concerned mainly with the physiological factors associated with bloat. At Davis, where he has been a member of the animal husbandry faculty since 1952, the scientist has been conducting mineral metabolism research.

Boda will return to the Davis campus in June of 1961.

Southern Illinois Note

H. H. OLSON, Associate Professor of Animal Industries, Southern Illinois University, Carbondale, has been selected as a participant in a workshop on the teaching of undergraduate physiology sponsored by the American Physiological Society. The workshop will be held at Carleton College, Northfield, Minnesota, August 8-20.

Training Program for Endocrinology

The University of Wisconsin Graduate School at Madison announces a postdoctoral training

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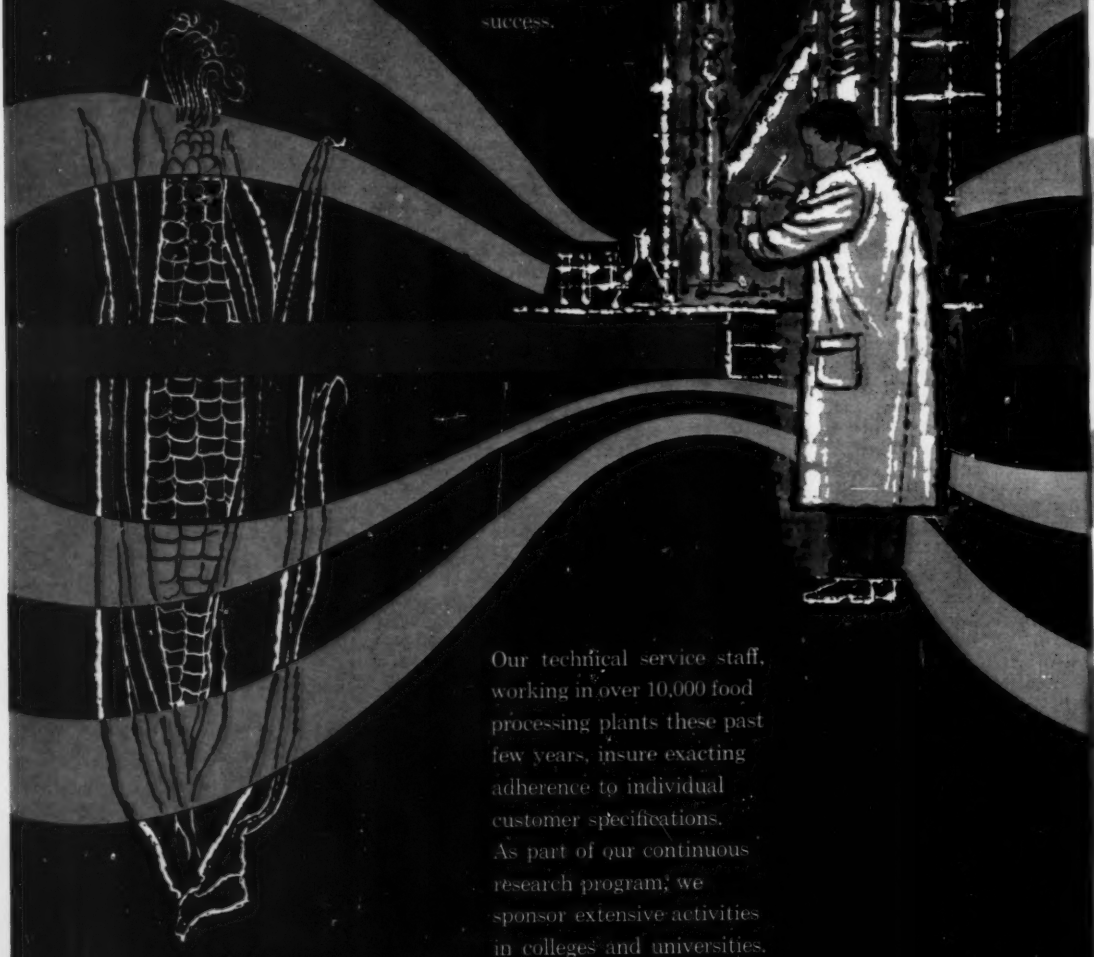

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program in endocrinology which began July 1, 1960. Its purpose is to increase the qualified personnel for teaching and for carrying on research in endocrinology.

Breadth of training will be emphasized. It consists of an integrated program of laboratory research, seminars, colloquia, work shops, and teaching under the coordinated auspices of faculty members from the Colleges of Letters and Science, Medicine, and Agriculture.

Candidates for postdoctoral fellowships (M.D. or Ph.D.) will be selected on the basis of professional promise and career interest. Appointments will carry a yearly stipend of $6,500. Send inquiries and requests for applications to: Dr. W. H. McShan, Birge Hall, University of Wisconsin, Madison 6, Wisconsin.

Borden's News

R. R. MINKLER has been appointed executive vice-president of the Borden Company's Central Division, which covers milk and ice cream operations in five states and food products sales in nine states. Also announced was the formation of the Chicago Metropolitan Region.

Creation of the Chicago Metropolitan region follows the division's consolidation of fluid milk and ice cream operations in Chicago and northern Illinois. The new region will be responsible for production and distribution of Borden's milk and ice cream products and also distribution of the company's full line of food products, including a variety of cheeses. H. A. SOLDWEDEL will be general manager.

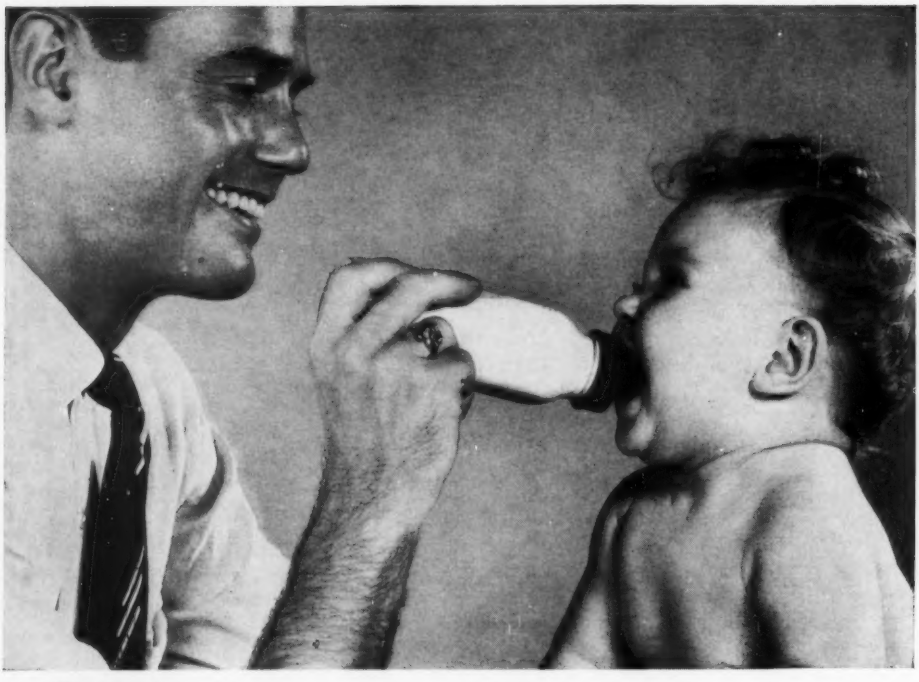
Soldwedel was formerly general manager of the company's Chicago fluid milk operations and has been with Borden's since 1955, when his family's Pekin, Illinois, dairy joined the company. In his new post he will be assisted by W. J. JENSEN as assistant general manager in charge of sales and W. L. HEDIN as assistant general manager in charge of operations. Jensen has been with Borden's since 1950 and Hedin since 1926.

Beatrice Foods Promotion

J. F. HAZELTON, JR., 4 Meadow Lane, Muncie, Indiana, has been promoted to district manager of the Indiana–Southern Ohio District of Beatrice Foods Company.

Hazelton succeeds LEWIS KOMMINSK, of New Bremen, Ohio, who has resigned as vice-president and district manager of Beatrice Foods for reasons of health after 25 yr. of service with the company. He will continue as manager of the Beatrice Foods Company plant in Muncie, 213 W. Seymour Street, where he will maintain his headquarters.

A native of Muncie and an Air Force veteran, Hazelton started with Beatrice Foods as an ice cream route driver and part-time salesman following his graduation from Indiana University, where he earned a Bachelor of Science degree in marketing.



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C. L. Wilcox Named Extension Dairyman at University of Minnesota

C. L. WILCOX, Archer, Idaho, has been named extension dairyman at the University of Minnesota, beginning July 1. His appointment was approved by the Board of Regents and will fill one of the vacancies left by the retirement of HAROLD R. SEARLES and RAMER LEIGHTON, long-time extension dairymen on the St. Paul campus.

C. L. Wilcox

Wilcox will cooperate with farmers, other extension specialists, county agents, and local Dairy Herd Improvement associations in educational programs on improved dairy practices.

He received his Ph.D. degree in dairy husbandry at the University in December, 1959, and recently has been teaching and conducting studies of dairy herds in several state institutions.

He has been at the University since 1955, doing graduate work and research. Since 1956, he has also been a part-time animal hubandman for the U. S. Department of Agriculture.

Before coming to Minnesota, he served in the U.S. Army in 1946-47 and 1951-53; earned his B.S. in dairy husbandry at Utah State University in 1951; and was an artificial breeding technician in Utah from 1953-55. He is married and has two children.

News from General Mills

DANIEL MERRILL has joined the staff of the General Mills, Inc., Central Research Laboratories, Minneapolis, Minnesota. He will serve as project leader in the Food Processing Department.

Dr. Merrill holds a bachelor's degree from the University of Minnesota and a doctor's degree from the University of Wisconsin.

A member of Alpha Zeta, Gamma Sigma Delta, and the American Dairy Science Association, Dr. Merrill came to General Mills from the North Star Dairy, St. Paul, where he served as a technical director.

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LETTERS TO THE EDITOR

I am writing to you to express my hearty approval of the new JOURNAL feature, Interpretive Summaries. We find them very useful in obtaining quickly the content of each article within its proper context, and also in explaining to nontechnical personnel the meaning of certain articles of interest.

I certainly hope that the JOURNAL will continue to publish these summaries.

JOHN H. NELSON, Director of Research
Dairyland Food Laboratories, Inc.
Waukesha, Wisconsin

For some time our staff has been discussing the new section in the JOURNAL OF DAIRY SCIENCE, the Interpretive Summaries. To come directly to the point, most of our staff are raising some very serious questions regarding this new section.

I feel that all of these can be summarized very briefly, in that the Association is going to have to make a decision whether the JOURNAL OF DAIRY SCIENCE is going to remain a purely scientific publication or whether it is going to become a trade journal. Perhaps we are a little biased in our opinion, but as long as we are a research group we do not feel we have any choice other than becoming even a stronger research publication. Basically, our men do not believe the Interpretive Summaries are necessary, and were simply established to patronize the trade people, and they do not feel this is the purpose of the JOURNAL OF DAIRY SCIENCE. This does not say that the various trades related to our industry do not need to be informed of our scientific work, because this is not true, but we are saying that we do not feel the JOURNAL OF DAIRY SCIENCE is the place to do this. Possibly, this means another trade publication or, possibly, individual stations should handle this through other outlets. Nevertheless, we felt led to pass on to you some of our thinking.

C. A. LASSITER, Head, Department of Dairy
Michigan State University, East Lansing

I have received a copy of the Supplement of the JOURNAL OF DAIRY SCIENCE in which the papers of the Fourth Biennial Symposium on Animal Reproduction are published. The printing and illustrations are excellent and the format distinctive.

I feel that the Supplement is a very commendable accomplishment.

R. H. DUTT
Professor of Animal Husbandry
University of Kentucky, Lexington

My copy of the proceedings of the Fourth Biennial Symposium on Animal Reproduction arrived last week. I have no criticisms of this

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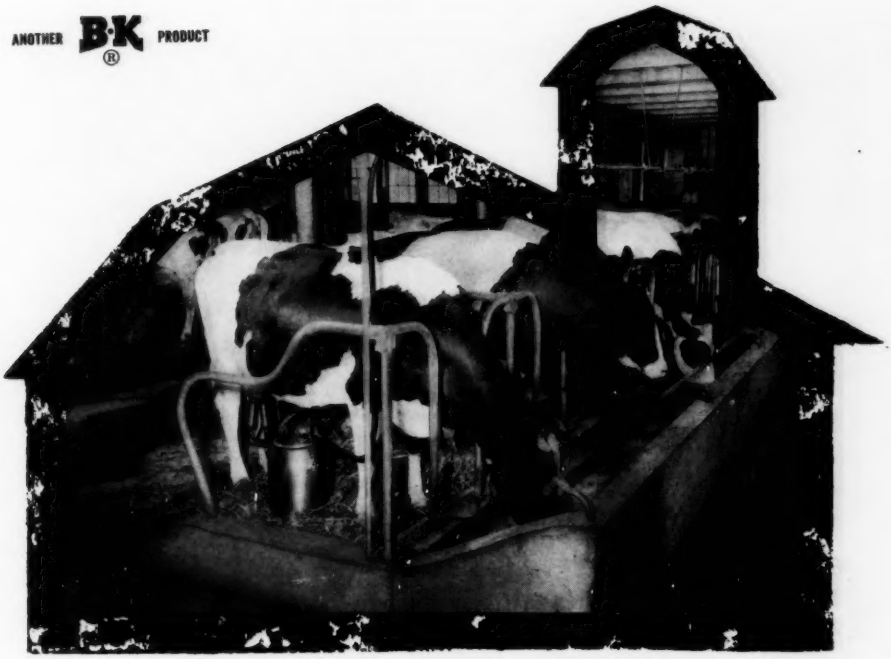

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supplement and only wish to commend you for undertaking this project.

As long as such a project is successful financially, I see no reason why you should not undertake future projects of this type. I have heard no constructive criticisms (or the other type, either) from any of the folks in the department.

J. O. ALMQUIST
Department of Dairy Science
The Pennsylvania State University
University Park

I can only say that a masterful job was performed in publication of the Symposium. The quality of printing and clarity of illustrations are not exceeded elsewhere. The format and organization of papers are also top level.

Only one question is aroused: Could the blank pages have been circumvented? For example, pages 32, 33, 36, 37, 40, 41, 44, 45. It is a bit puzzling to find them, and aside from detracting from the professional nature of the publication, seems wasteful.

On all other counts you are to be congratulated.

SEYMOUR KATSH, Assistant Professor
Department of Pharmacology
University of Colorado Medical Center, Denver

The supplement to the JOURNAL OF DAIRY SCIENCE, Volume 43, April, 1960, is a very valuable and true summary of the proceedings held on June 18-20, 1959.

I feel rather strongly that this type of supplement, in which all the information in a given field of interest is gathered in one place, is much easier to read and also makes a much handier reference than when the articles are scattered through several months of the regular issues of a journal.

ROBERT W. NOYES, M.D.
Department of Obstetrics and Gynecology
Stanford University School of Medicine
Palo Alto, California

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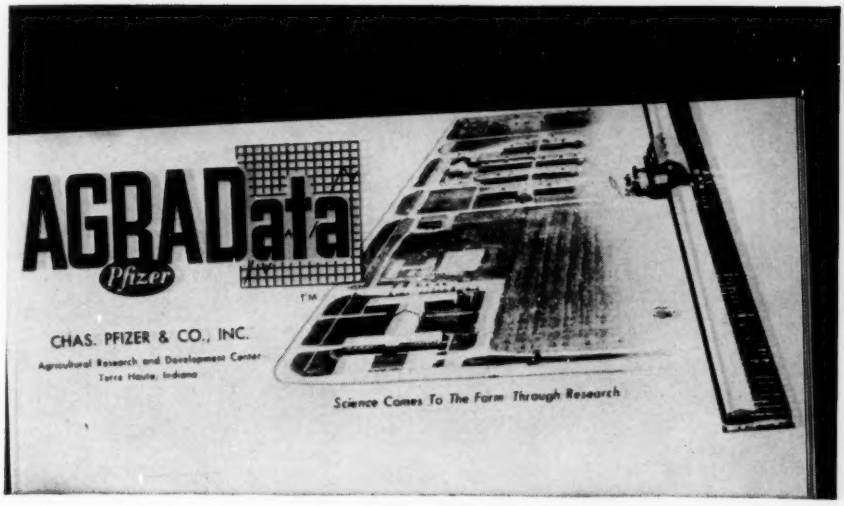

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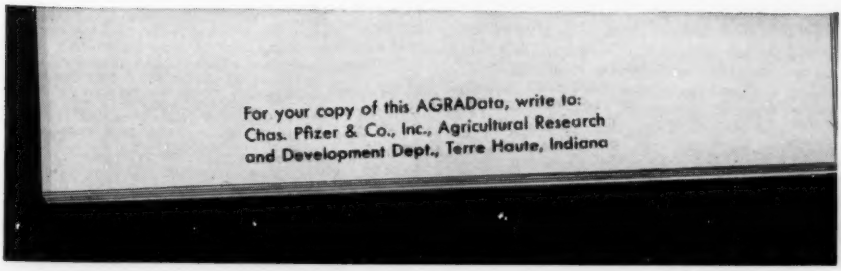
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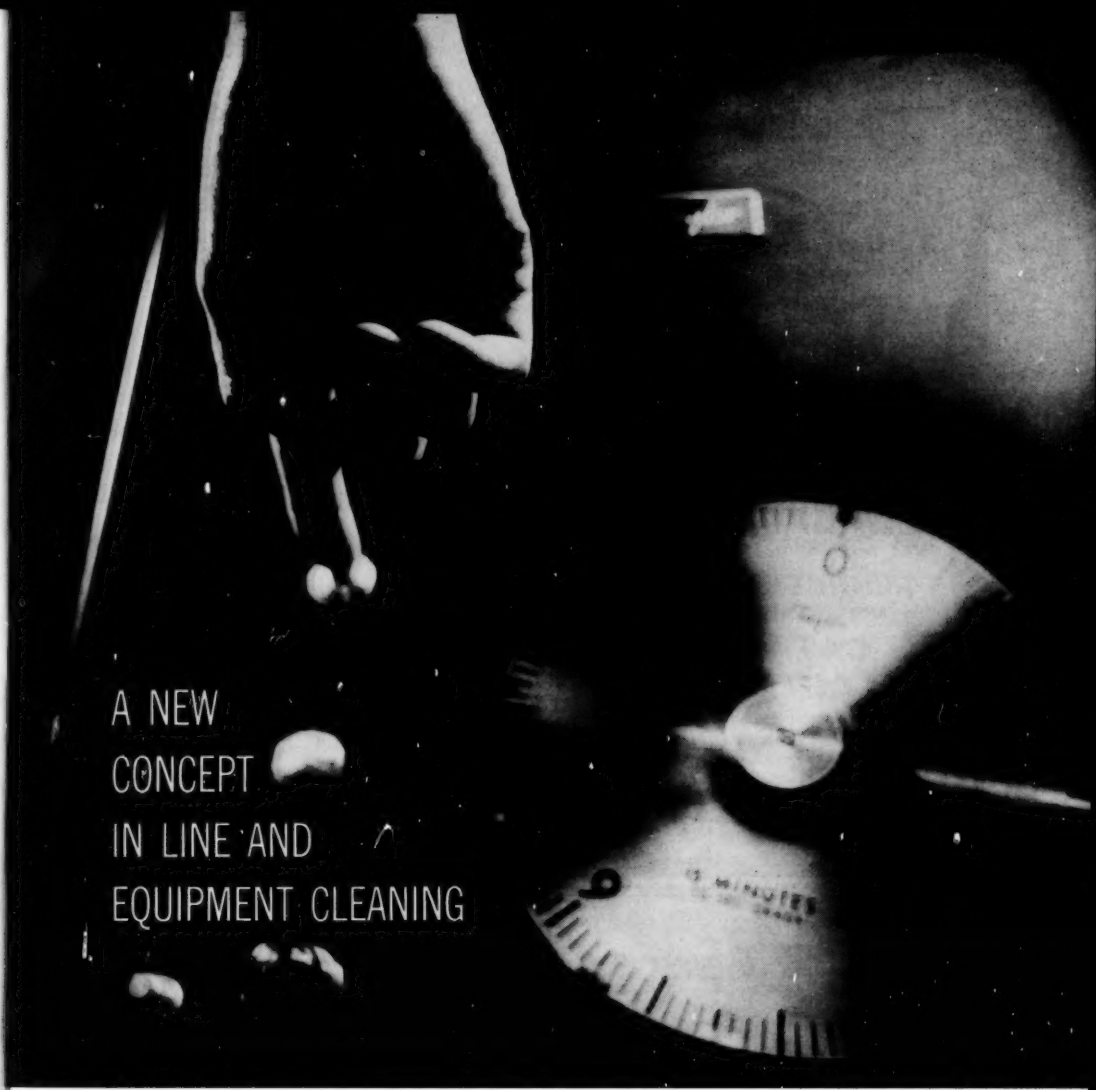

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QUANTITATIVE DETERMINATION OF CARBOHYDRATES IN ICE CREAM BY PAPER CHROMATOGRAPHY[1]

T. C. CHOU[2] AND J. TOBIAS

Department of Food Technology, University of Illinois, Urbana

SUMMARY

Sugars in ice cream were separated by descending paper chromatography, using an irrigation solvent system consisting of *n*-butanol:ethanol:water (2:1:1 v/v). Paper strips containing the resolved sugars were eluted with water. Quantitative determinations were performed photometrically on aliquots of eluates reacted with anthrone reagent.

No preliminary treatment of the ice cream was necessary except dilution with water. The following sugars could be resolved on a single chromatographic strip: fructose, glucose, galactose, sucrose, maltose, lactose, and higher sugars. Four sugars, glucose, sucrose, maltose, and lactose, formed distinct spots when an ice cream containing corn syrup was analyzed. Recoveries ranging from 93 to 104% and averaging close to 100% indicate a fair degree of reliability of the technique.

Ice cream may contain as few as two major sugars such as sucrose and lactose, but frequently contains solids derived from corn syrup containing nine or more different sugars (13). Still other sugars, such as fructose or galactose, also may be present.

Until the advent of chromatography and its application to carbohydrate analysis (4, 10, 12, 13), a quantitative determination of sugars in ice creams of unknown composition was extremely difficult, as the reduction tests failed to differentiate between the many sugars present.

A technique for the separation of corn syrup components was developed by Whistler and Hickson (13). A chromatographic test for lactose in milk was reported by Honer and Tuckey (9). Helbert and Brown (7) have made extensive studies on the application of the anthrone reaction to the quantitative determination of carbohydrates and found that under suitable experimental conditions sugars could be determined with great precision. Fagen *et al.* (3) reported on a quantitative determination of lactose using anthrone. Haas and Fleischman (5) found no interference of tannic acid and amino acids in the anthrone test for carbohydrates in beer.

These investigations indicate a definite possibility for the successful separation of sugars in ice cream by paper chromatography. The purpose of this study was to develop such a procedure and quantitatively determine the sugars present.

Received for publication January 21, 1960.

[1] The data published here are taken from a thesis submitted by the senior author in partial fulfillment of the requirements for the degree of Master of Science.

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ANALYTICAL METHOD

Separation. The melted ice cream samples were diluted with distilled water to make approximately a 30% solution by weight. The solutions were spotted on Whatman No. 1 chromatographic grade filter paper whose original dimensions were 18.25 by 22.5 in. The papers were prepared by tracing along the heavily outlined edges of a template, shown in Figure 1.

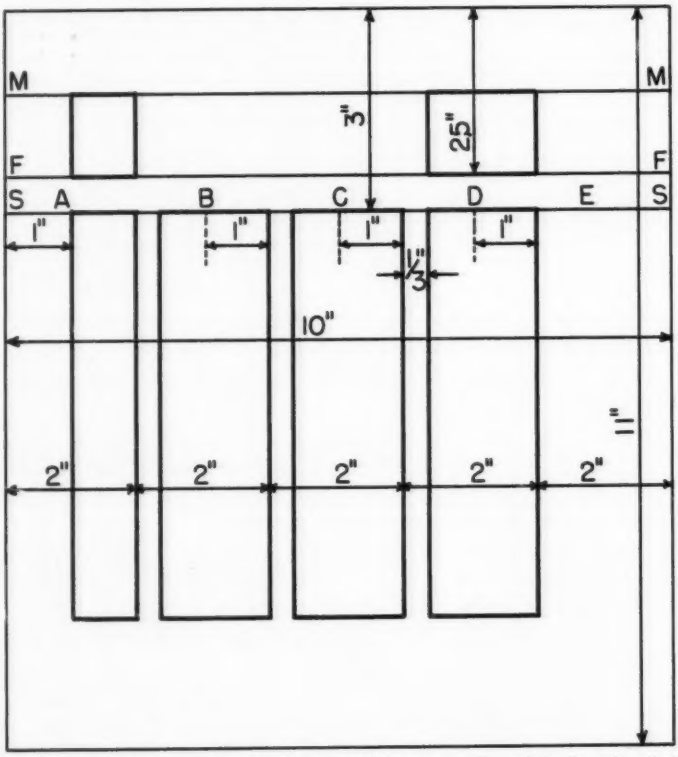


FIG. 1. Template for marking chromatographic paper. Template length of 11 in. is arbitrary.

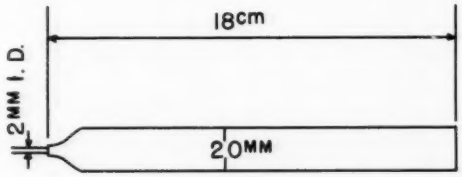
The sugar-containing solutions were applied at positions marked A, B, C, and D on the starting line 3 in. from the end of the strip. The spots (3 to 5 mm. in diameter) were dried with an air blower. The strips were folded back at Line F-F and hung over the antisiphon rod of the solvent tray assembly in the Chromatocab (Research Equipment Corp., Oakland, Calif.). An anchor rod was placed in the first fold (M-M), supporting the strip so that the lower end hung free. Four to eight strips were developed at one time. The strips were equilibrated for periods of 6 to 24 hr. against an atmosphere saturated with the irrigating solvent. The troughs were then filled with a solvent mixture

consisting of *n*-butanol-ethanol-water (2:1:1 v/v), and the Chromatocab was closed. One hundred to 115 hr. were allowed for development. The length of development time was determined by periodic removal of single reference strips from the Chromatocab, followed by color development of the known sugar spots. The strips were removed from the Chromatocab by means of the anti-siphon rod, and allowed to air-drip for about 1 hr. under a ventilated hood. They were supported by stainless steel clips.

Quantitative determination. The A and C guide strips were cut off and uniformly sprayed with aniline phthalate indicator solution. When dry, they were heated at 136° C. in a hot air convection oven for 10 to 15 min. to complete the color reaction. The E strips were used as blanks.

The A and C strips were aligned with the B, D, and E strips. Straight lines were drawn between spots across the entire width of the paper to mark the presumed location of sugars for quantitative determination.

The sugars were identified by comparison with the developed A and C strips and the areas containing them were cut from the B and D sections. A corresponding area of paper from the same relative location on the blank strip E was also cut out. Each excised area was cut into 0.5-in. squares and placed in a 100-ml. beaker, avoiding contact with the fingers as much as possible. Twenty-five milliliters of distilled water was pipetted into the beaker and the papers were extracted for 25 min., stirring occasionally with a glass rod. The mixture was filtered through a dry glass wool plug in a filter tube as shown in Figure 2. The filtrate was collected in a 50-ml. Ehrlenmeyer flask.



FILTER TUBE

FIG. 2. Tube used for filtering sugar eluates.

Quantitative determinations were made by means of the anthrone reaction (1, 7, 11). Two milliliters (or 4 ml., depending on the carbohydrate content) of the filtrate was pipetted into an 18- by 150-mm. Pyrex glass test tube. The solution was evaporated to dryness at 70–90° C. in a vacuum evaporating oven. The tubes were removed from the oven and cooled to room temperature. Two milliliters of 27.5 *N* H_2SO_4 was added to dissolve the sugar. After 10 min., 4 ml. of anthrone reagent was added and, after mixing, the tubes were covered with aluminum foil. Because 27.5 *N* H_2SO_4 was the solvent for both sugar and anthrone, no heat of mixing was evolved. Test solutions were heated in a thermostatically controlled water bath at 90° C. for 20 min. After heating, the

samples were quickly transferred to a cold water bath ($4 \pm 1°$ C.) for 3 min. Heating and cooling times were measured to ± 2 sec. The test tubes containing the reacting solutions were spaced in wire racks in the heating and cooling baths to facilitate uniform heat transfer. After tempering for 30 min. at room temperature ($23 \pm 5°$ C.), the per cent transmission at 625 mμ was determined on a Model 11 Coleman Universal spectrophotometer. Test samples were always protected from direct sunlight. The sugar concentrations were determined by reference to the corresponding standard curve.

Reagents were prepared as follows: The *n*-butanol and ethanol were refluxed with 2,4-dinitrophenylhydrazine (12 g. per liter) for 4 hr. and then distilled. The aniline hydrogen phthalate reagent was prepared by adding 0.93 g. aniline (colorless) and 1.66 g. phthalic anhydride to 100 ml. water-saturated *n*-butanol and mixing thoroughly to get a clear solution. The 27.5 *N* H_2SO_4 was prepared by adding 1,000 ml. of reagent-grade H_2SO_4 (95-98% H_2SO_4) into 480 ml. distilled water, and its normality was checked by titration against standard sodium hydroxide solution. The anthrone solution was prepared by dissolving 0.16 g. of anthrone in 100 ml. of 27.5 *N* H_2SO_4, allowing about 60 min. to effect complete solution. The reagent was always freshly prepared and discarded when 24 hr. old.

Preparation of standard curves. Solutions of glucose, maltose, sucrose, lactose, and fructose were prepared by weighing 2.0000 g. of the respective sugars, dissolving in distilled water, and making up to 100 ml. With the exception of maltose, all sugars were of reagent-grade quality. They were dried for at least 2 wk. in a calcium chloride desiccator. Lactose was dried to constant weight by heating to 80° C. in a vacuum oven. Maltose was purified by double recrystallization. Its optical rotation in 10% solution was $[\alpha]_D^{21} = 130.4$.

Quantities of from 5 to 25 μl. of each sugar solution were spotted in duplicate and the papers were developed for 10 hr. in the Chromatocab. The sugars were eluted from the chromatographic paper, reacted with anthrone reagent, and the color was measured photometrically. A least-squares equation for the best-fitting straight line was established for the standard curves of all the sugars. Standard curves are shown in Figure 3. It is to be noted that the concentration intervals are 8, 16, 24, 32, and 40 μg. If the amount of sugar spotted is 100 μg. and it is eluted with 25 ml. water, a 2-ml. aliquot of the eluate will contain 8 μg. of sugar. The per cent transmission plotted in Figure 3 corresponds to the quantity of sugar in the aliquot of eluate on which a photometric determination was made.

Resolution of a mixture of sugars in aqueous solution. The distance of travel of various sugars in relation to the distance traveled by glucose, Rg, was determined by using 5-μl. quantities of 2% solutions of lactose, maltose, sucrose, and fructose, and a high conversion type of corn syrup. Results are given in Table 1. A chromatogram of nearly all sugars which may be encountered in ice cream is shown in Figure 4.

Recoveries from mixed aqueous solutions of sugars. An aqueous solution was prepared containing 3.0000 g. sucrose, 6.0001 g. lactose, 2.0050 g. glucose,

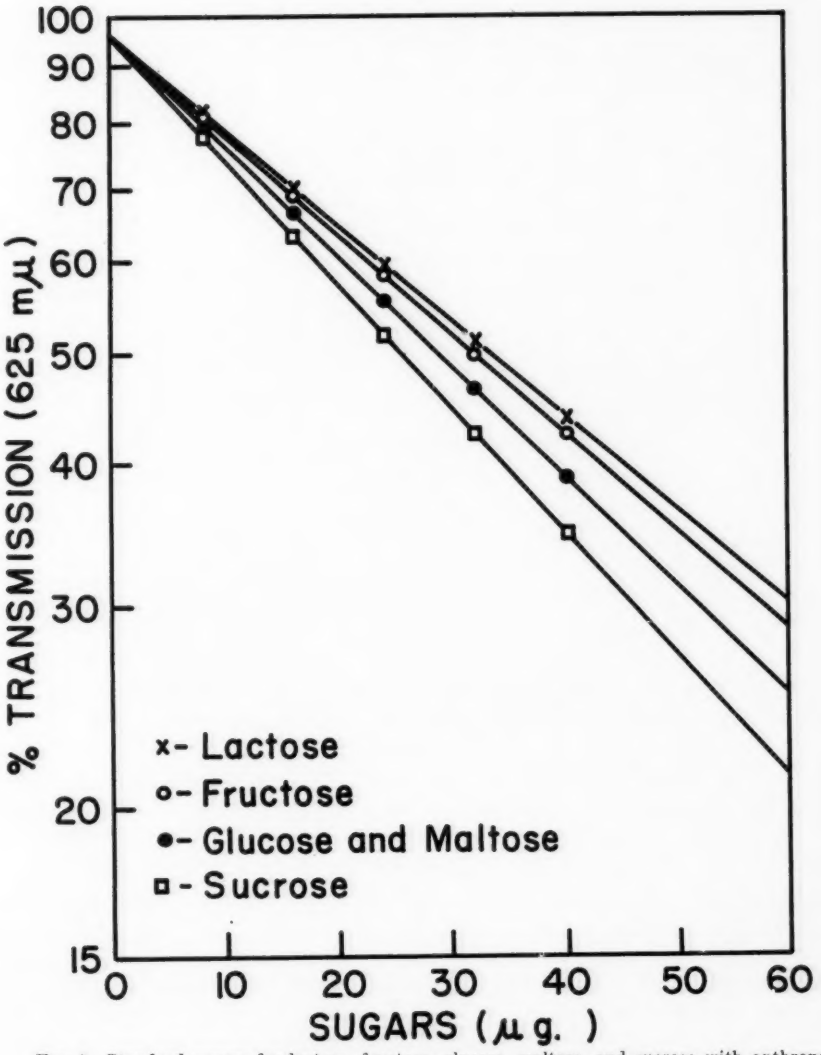


FIG. 3. Standard curves for lactose, fructose, glucose, maltose, and sucrose with anthrone reagent.

and 1.0020 g. maltose in 100 ml. of solution. Five-microliter quantities were spotted on chromatographic paper and recoveries of each sugar were determined following development. Results are given in Table 2.

Recoveries of sugars from ice cream of known composition. Ice cream mixes were prepared to contain 12% fat, 11% milk-solids-not-fat, 0.3% stabilizer, and varying amounts of sugars, using cream (40.0% fat) and condensed skim-

TABLE 1

Relative mobilities of sugars

Components	Sugars[b]	R glucose[a]
1	Fructose	1.162
2	Glucose	1.000
3	Galactose	0.900
4	Sucrose	0.675
5	Maltose	0.503
6	Lactose	0.400
7	Isomaltose	0.393
8	Maltotriose	0.269
9	Isomaltotriose	0.222
10	Maltotetraose	0.156
11	Maltopentaose	0.145
12	Maltohexaose	0.080
13	Maltoheptaose	0.040

[a] Solvent *n*-butanol:ethanol:water (2:1:1 v/v). Temperature 23-30° C. Whatman No. 1 filter paper. Descending technique.

[b] Identification of corn syrup components was made by comparison with Rg values found in the literature.

TABLE 2

Recovery of sugars from a mixed aqueous solution

Amount of sugar in aliquot of eluate	Amount of sugar recovered	Recovery
(μg.)	*(μg.)*	*(%)*
	Glucose	
8	7.7	96.0
8	7.6	94.8
8	7.7	96.0
8	8.3	103.7
	Lactose	
24	23.1	96.3
24	23.4	97.5
24	23.1	96.3
24	22.8	95.0
	Maltose	
4	3.97	99.3
4	3.97	99.3
4	3.97	99.3
4	3.97	99.3
4	4.07	101.8
8	7.6	95.0
8	8.1	101.3
	Sucrose	
32	30.1	94.1
32	30.1	94.1
32	32.3	100.9
32	32.2	100.6
32	29.7	92.8
32	31.2	97.5
32	32.3	100.9
32	32.3	100.9
52	48.3	93.0
52	48.3	93.0
Average		97.5
		$\sigma = \pm 3.17$

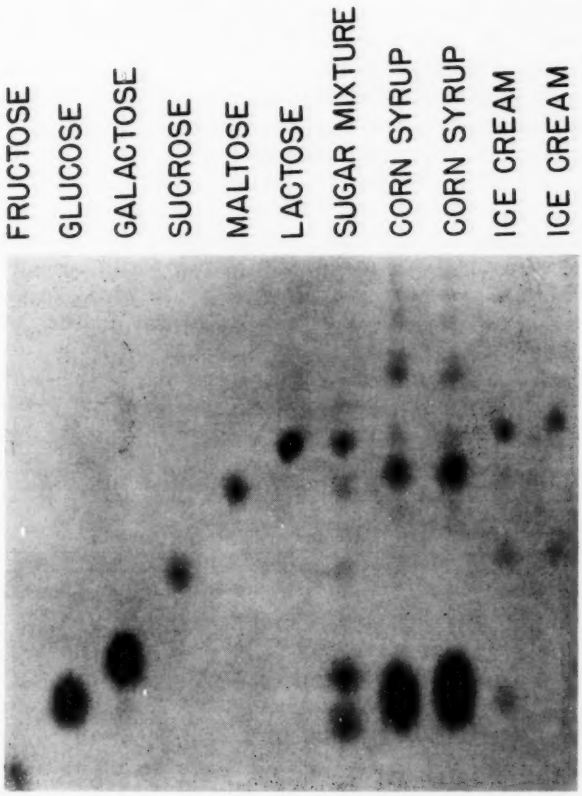


FIG. 4. Chromatogram of aqueous solutions of single sugars, mixture of sugars, corn syrup, and ice cream.

milk (35.0% milk-solids-not-fat) as ingredients. Mix 1 contained 11.5% sucrose, 2% glucose, and 2% fructose; Mix 2 contained 14.5% sucrose; and Mix 3 contained 12% sucrose and 4% corn syrup (Sweetose). Quantities of ice cream mixes ranging from 26.6 to 28.85 g. were diluted to 100 ml. with distilled water. Ten microliters (or 5 μl) of each sample was spotted and the sugars were determined following development and elution.

To determine the lactose content of the mixes, a modification of the Hinton and Macara (8) procedure was used as an independent test to check against the chromatographic procedure. The determination was carried out on the condensed skimmilk and cream.

The corn syrup was analyzed by the same chromatographic procedure as the ice cream mix. A quantity of 7.7723 g. of corn syrup was dissolved in distilled water and made up to 100 ml. Ten microliters of the solution was applied on the chromatographic paper, which was developed for 76 hr. at 25° C. The

sugar components were eluted and determined with the anthrone reagent. The total dry solids of the corn syrup were determined by the Evans and Fetzer method (2). Results are given in Table 3.

In ice cream containing corn syrup, the entire area of the strip above lactose, and including the point of application, was excised and the sugar determined as one component, termed maltopolyose.

DISCUSSION

The irrigating solvent, *n*-butanol:ethanol:water (2:1:1 v/v), was suggested by Whistler and Hickson (13). An examination of Figure 4 indicates that a satisfactory separation was obtained with most of the sugars which may be encountered in ice cream. The Rg values recorded in Table 1 further confirm this finding.

The separation of glucose from galactose may not be sufficiently sharp for quantitative work of highest accuracy, but galactose would only infrequently

TABLE 3
Determination of sugars in ice cream of known composition

Sugar	Added[a]		Recovered	Added	Recovered
	(μg.)			(%)	
			Ice Cream 1		
Lactose	6.25		6.3	5.40	5.46
Lactose	6.25		6.3	5.40	5.46
		Av.	6.3		5.46
Sucrose	13.25		13.3	11.50	11.53
Sucrose	13.25		13.3	11.50	11.53
		Av.	13.3		11.53
Glucose	2.3		2.4	2.00	2.08
Glucose	2.3		2.4	2.00	2.08
		Av.	2.4		2.08
Fructose	2.3		2.3	2.00	2.00
Fructose	2.3		2.3	2.00	2.00
		Av.	2.3		2.00
			Ice Cream 2		
Lactose	11.86		11.9	5.40	5.42
Lactose	11.86		11.9	5.40	5.42
		Av.	11.9		5.42
Sucrose	31.84		33.27	14.5	15.16
Sucrose	31.84		33.20	14.5	15.12
		Av.	33.24		15.14
			Ice Cream 3		
Lactose	11.5		10.9	5.40	5.12
Lactose	11.5		10.9	5.40	5.12
		Av.	10.9		5.12
Sucrose	25.5		23.8	12.00	11.20
Sucrose	25.5		24.1	12.00	11.33
		Av.	23.95		11.27
Maltopolyose	2.05		1.9	0.96	0.89
Maltopolyose	2.05		2.1	0.96	0.99
		Av.	2.0		0.94
Maltose	2.5		2.5	1.17	1.17
Maltose	2.5		2.5	1.17	1.17
		Av.	2.5		1.17
Glucose	2.7		2.7	1.26	1.26
Glucose	2.7		2.8	1.26	1.32
		Av.	2.75		1.29

[a] Quantities of sugars in aliquots of eluate used in determination.

be found in ice cream in greater than trace quantities. Maltose and lactose, if present in high concentrations, also may be difficult to separate. In the concentrations normally found in ice cream, however, no particular difficulty was encountered, as seen in the recovery data in Tables 2 and 3.

A greater degree of separation of maltose from lactose may be obtained by longer development or by selection of a different irrigating solvent. An ascending technique was successfully attempted in which a solvent system was employed consisting of isopropanol:pyridine:water:acetic acid (8:8:4:1 v/v).

The rate of movement was found to be affected to a considerable degree by temperature. Physical facilities did not permit the performance of all experiments under constant temperature, which made it necessary to watch the papers carefully to prevent the fastest-moving components from going off the paper. If the chromatographic process was stopped too soon, some of the sugars, particularly maltose and lactose, did not completely resolve.

A disadvantage of this procedure is the length of time, usually about 100 hr., required to obtain resolution. It is possible that by a selection of a different solvent system or a different technique, such as the horizontal technique of Roberts (10), the time required to complete an analysis may be shortened. There was a sufficient number of points in favor of the present procedure, however, to justify its adoption. Ice cream could be analyzed with no preliminary treatment except for dilution with water, and practically all sugars normally encountered in ice cream could be determined on a single strip of chromatographic paper. Recoveries ranging from 93 to 104% and averaging fairly close to 100% spoke well for the selection of this technique, as did a satisfactory reproducibility. The procedure may not be termed as a simple one, but it is no more involved than other microanalyses which employ chromatography for separation of components.

Under certain conditions, which could not always be predicted, the spots on a given paper did not travel with an even front. This caused considerable difficulty in selecting the area on the strip which was to contain the sugar to be analyzed. Much of this difficulty was eliminated by marking the paper by means of the template shown in Figure 1. This provided for a locator strip at the edge, as well as in the center, of the paper.

Several indicator spray reagents were tried for marking the locator strips. Ammoniacal silver nitrate was found sufficiently sensitive, but its use was discontinued because after a short time the entire paper turned dark brown.

A spray reagent consisting of o-aminodiphenyl was found satisfactory. Timell *et al.* (12) reported that 0.1-μg. quantities of sugars could be detected by this reagent. The spray reagent employed throughout most of the present studies was aniline phthalate. Its sensitivity was satisfactory when the sprayed chromatograms were heated at 135° C. for 15 min. With lower heating temperatures, maltose, sucrose, fructose, and other oligosaccharides appeared with only a faint spot, or none at all.

The filter tube seen in Figure 2 proved to be quite effective in facilitating elution of sugars from the chromatographic paper. The tightly packed glass wool plug appeared to prevent the passage of filter paper fibers which would have interfered in the photometric determinations. The procedure was selected in preference to other extraction techniques employing reflux condensing, because the latter were not as simple. The filter tubes could be used without repacking each time, but considerable error could result from using tubes which were not properly washed and dried. Drying at 140° C. for 72 hr. was found to be essential.

It is not known whether complete elution of the sugars is obtained by this procedure, but since the standard curves were prepared from data obtained after chromatographic separation of known quantities of sugars and elution from the paper, it is only necessary that the same fraction of sugars be eluted each time. Recovery data indicate that this was the case.

Several quantitative procedures were considered before adopting the anthrone reagent technique. The micro-ferricyanide technique of Hagedorn and Jensen (6) was found to yield accurate and reproducible results for glucose, but considerable developmental work would have been required to assess reducing values of inverted sucrose, fructose, lactose, and galactose. The o-aminodiphenyl procedure, it was felt, possessed no advantages over the anthrone method, except for the possibility of eliminating the locator strips.

The anthrone technique was considered the most desirable because no preliminary treatment of the sugars was necessary, excellent sensitivity and reproducibility were obtained, and a single standard curve served both glucose and its oligosaccharides. Haas *et al.* (5) reported that the color developed by the reaction of anthrone with hexoses is not interfered with by amino acids and certain other substances. It was thus felt that this reagent would be reasonably safe from the standpoint of interferences when used in connection with ice cream which was not deproteinized or defatted.

The optimum concentration of sugars in the aliquot used for color development was found in the range of 4 to 40 μg. Below 4 μg. the values are of the same order as the paper blank and above 40 μg, incorrect recoveries are obtained. No difficulty was generally encountered in obtaining such concentrations, as quantities ranging from less than 1 ml. to over 20 ml. of eluate may be used for the determination. In the usual case, 2 to 4 ml. of the eluate was found to give the desired concentration.

Oligosaccharides reported in Table 1 were components of corn syrup. Presumptive identification of these components was made by comparison of Rg values found in the literature (13).

Ice Cream 3 in Table 3 is reported to contain a quantity of maltopolyose. This term was selected to describe all the components of corn syrup found above maltose on the chromatogram. The concentration of these components in ice cream is too low to form distinct spots on the locator strips and for that reason the entire area above lactose, including the point of application, was excised and determined as one component.

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A SIMPLIFIED METHOD FOR THE DETERMINATION OF IODINE^{131} IN MILK

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SUMMARY

A simplified method has been developed for the determination of iodine^{131} (I^{131}) in milk. This method is suitable for surveillance purposes and can be employed in conjunction with the strontium^{90} determination described by Murthy *et al.* (12). It is based on the gross γ-counting of proteins obtained by trichloroacetic acid precipitation of milk preserved with formaldehyde. Formaldehyde favors a reaction wherein the inorganic form of I^{131} present in milk is transformed into protein-bound I^{131}. Work is under way to determine the mechanism of this reaction, which is not clearly understood. The method is simple and accurate, and results are comparable to those obtained by gamma spectrometric methods (2). It involves no instruments other than a shielded 2-in. solid NaI (thallium-activated) crystal with a photomultiplier assembly fed through a cathode follower and coupled directly to a decade scaler. These instruments ordinarily are available in any radiochemical laboratory. The recovery of I^{131} is $98 \pm 2\%$ and the presence of γ-emitting K^{40}, Cs^{137}, and $Ba^{140} + La^{140}$, usually found in milk, has no effect on the I^{131} determination.

Radioactive iodine is a fission product of nuclear weapons tests or of reactor operations. During fallout it is distributed in the biosphere, in soil and plants, and in animals and man (4). Radioactive iodine from air, water, and pasture contaminated with fallout of recent origin is accumulated in the thyroid (15) of a grazing animal and, when metabolized, part of it is secreted into milk. The presence of I^{131} in milk due to fallout (3) and reactor accidents (6) has been observed. Tracer studies show that of the administered dose, less than 10% appears in cow's milk (7, 11), while as much as half can be recovered in the milk of rabbits and goats (19).

Of the several isotopes formed as a result of nuclear fission, only $I^{131\text{-}135}$ have been considered significant in the thyroid uptake. With the exception of I^{131}, all the other isotopes have short half-lives and decay out rapidly. Their presence may be observed in the vicinity of the test site immediately after weapons testing (5). However, available data on radioactivity of thyroids of cattle and lamb collected from different parts of the United States strongly suggest that the radioactivity is due to I^{131} (15).

Iodine^{131} may be present in milk in the inorganic form as the iodide or it may be bound with the proteins. Tracer studies have shown that it appears mostly in the inorganic form in the milk of man (10), goats, and rabbits (19); in dogs (16) and lactating rats (14); however, 30-60 and 86%, respectively, is protein-bound. In milk from cows injected with a single dose of carrier-free I^{131}, all the iodine was in inorganic form (7), while in milk from cows dosed continuously with I^{131} approximately 81.7%-91.4% was in the inorganic form, 4.7%-13.1% was protein-bound, and less than 0.1% was associated with the fat.

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The distribution of I^{131} between inorganic and protein fractions was affected by the amount of stable iodine in the cow's diet (11). Therefore, to develop chemical methods, the distribution of I^{131} in milk and its fractions must be taken into consideration in order that total I^{131} may be determined.

Several chemical (1, 9) and gamma-ray spectrometric methods (8, 17) have been developed for I^{131} determination in cow's milk. In the chemical methods, time-consuming steps necessary to eliminate interfering substances include sample preparation, solvent extraction, and a series of oxidation-reduction reactions, before the iodine is precipitated as silver iodide for determining its activity.

Gamma-ray spectrometric methods are simpler, requiring no sample preparation and providing considerable savings in time. An appropriate amount of milk sample is transferred to a container, placed over a NaI (thallium-activated) crystal, coupled to a photomultiplier tube and to a single or a multichannel analyzer. The I^{131} activity is measured by observing the counting rates at 364 Kev. However, corrections must be made for the effects of K^{40}, Cs^{137}, and Ba^{140} + La^{140} on the I^{131} photo-peak. These involve tedious mathematical calculations.

Because these procedures require pulse-height analyzers and constant maintenance and, therefore, are expensive, a simpler method was desired for use in conjunction with other radiochemical analyses, requiring no instruments other than those ordinarily available in radiochemical laboratories. An investigation of these possibilities has resulted in the simplified method described here.

MATERIALS AND METHOD

Milk samples. All milk samples employed in this study, unless otherwise mentioned, were from cows dosed with 100 μc. of I^{131} and were obtained from the Dairy Science Department, University of Missouri, Columbia.

Procedure. Sample preparation by trichloroacetic acid (TCA) precipitation is the same as described by Murthy *et al.* (12). The proteins obtained from one liter of milk preserved with formaldehyde are washed three times by dispersing them in 250-ml. portions of 12% TCA. The washings are removed by centrifugation in a 250-ml. polyethylene bottle and discarded. The γ-activity due to I^{131} in the proteins is determined, using a shielded 2-in. solid NaI crystal with photomultiplier assembly fed through a cathode follower and connected directly to a decade scaler.

RESULTS AND DISCUSSION

Distribution of I^{131} in milk and its fractions. To determine the distribution of I^{131} in milk and its fractions, milk samples containing 10,000 to 30,000 $\mu\mu$c. I^{131} per 100 ml. of milk were divided into several portions and treated as follows:

(a) Same as described under Materials and Method. The filtrate and the washings were made up to 3.5 liters with distilled water.

(b) Milk was precipitated at pH 4.5 with diluted HCl. The casein was removed by filtration and was washed three times by dispersion in water. The filtrate and washings were diluted to 3.5 liters with distilled water.

(c) The milk was centrifuged at 40,000 r.p.m. in a Spinco Model E ultracentrifuge for 2 hr. The sedimented caseinates were washed two times with cold distilled water and the washings removed by recentrifugation. The supernatant and the washings were made up to 3.5 liters with distilled water.

The proteins obtained in a, b, and c steps were each dispersed in NaOH and diluted to 3.5 liters with distilled water.

An aliquot of the original milk diluted to 3.5 liters and the various fractions were counted on a gamma-ray spectrometer as described by Branson *et al.* (2).

An inspection of the data presented in Table 1 shows that approximately

TABLE 1

Per cent distribution of iodine^{131} in milk and its fractions

Fractions	Per cent distribution of I^{131}			
	Milk preserved with formaldehyde		Fresh milk	
	(1)	(2)	(3)	(4)
Whole milk	100.0[a]	100.0[a]	100.0[a]	100.0[a]
Proteins from TCA-pptd. milk	98.0	99.0	9.0	5.5
Protein-free filtrate from TCA-pptd. milk	1.5	1.0	91.0	94.0
Isoelectric casein from HCl-pptd. milk	96.0			
Casein-free serum from HCl-pptd. milk	3.5			
Native caseinate	95.0[b]			

[a] The level of I^{131} in milk ranged between 10,000 to 30,000 $\mu\mu$c. per 100 ml.
[b] Low value possibly due to incomplete sedimentation of caseinates.

98-99% of the I^{131} in milk is protein-bound, of which 95% is bound with the casein obtained either by isoelectric precipitation or by ultracentrifugation (native caseinate), and less than 2% is associated with the serum proteins. These observations, however, are contradictory to the data presented by Glassock (7) and Lengemann and Swanson (11), who showed that most of the I^{131} in milk is in the inorganic form as the iodide. Therefore, in seeking an explanation of this discrepancy, it was found that the 40% formaldehyde used as a preservative might have caused this phenomenon. Fractionation studies on fresh milk (Table 1) and observations of Ward (18) confirmed that formaldehyde promoted a reaction wherein all the inorganic form of I^{131} was transformed into an organic form, i.e., protein-bound I^{131}. Since the mechanism of this reaction is not clearly understood, work is in progress to find the explanation.

Effect of formaldehyde on the binding of I^{131} to proteins. To determine the amount of formaldehyde required to convert inorganic I^{131} into the protein-bound form, fresh milk containing I^{131} was divided into four 100-ml. portions. Forty per cent formaldehyde solution in the amounts of 0.05, 0.10, 0.15, and 0.20 ml., respectively, were added to each portion and the samples were allowed to stand at room temperature for 48 hr. After storage, the proteins were precipitated as described under Materials and Method and their γ-activity was determined in a gross γ-counter. The results (Table 2) show that the binding

TABLE 2

Effect of formaldehyde addition to milk on the protein-bound iodine[131] concentration

Milk	Formaldehyde	Protein-bound I^{131}	
		Milk 1	Milk 2
(ml.)		(%)	
100	0.00	9.1	5.5
100	0.05	104.6	101.3
100	0.10	99.2	99.7
100	0.15	100.8	101.1
100	0.20	104.7	101.4
		Av. 102 ± 2%	100.9 ± 0.8%

of I^{131} to proteins is independent of the amount of formaldehyde concentration at the levels tested and that the transformation takes place even at the low formaldehyde level of 0.05%.

Effect of time of storage of milk with formaldehyde on the binding of inorganic I^{131} to milk proteins. To determine the time required for conversion of inorganic I^{131} to protein-bound I^{131} in the presence of formaldehyde, several 100-ml. portions of milk with added 0.1 ml. of 40% formaldehyde were stored at room temperature (25° C.) for varying times. The proteins were separated

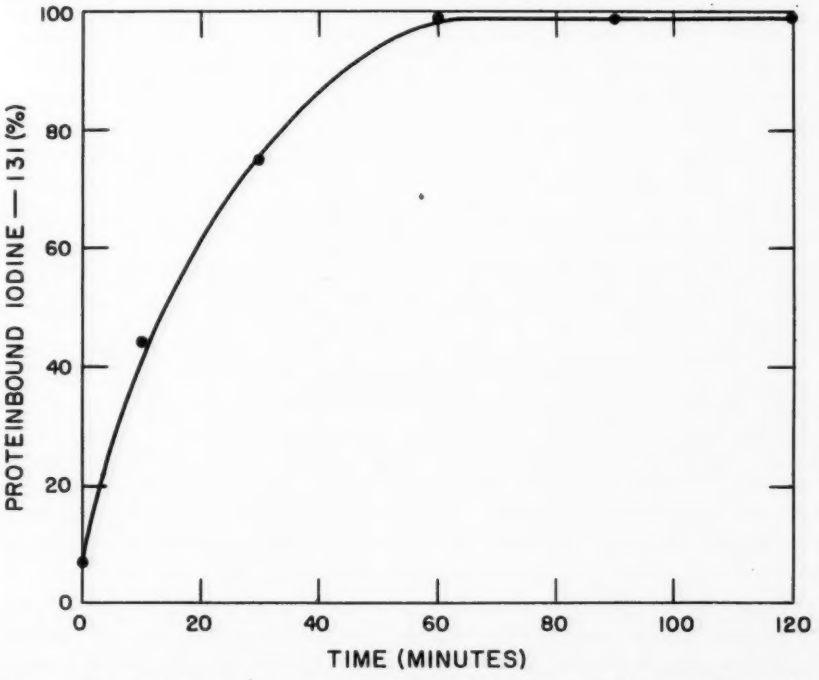

FIG. 1. Effect of storage time of milk with formaldehyde on protein-bound I^{131} content.

from the samples at intervals and their gross γ-activity was determined as before. The results presented in Figure 1 show that most of the inorganic I^{131} is transformed to protein-bound I^{131} within approximately 1 to 2 hr. after the addition of formaldehyde.

Adequacy of gross γ-counting of I^{131}. Since the present method was developed for the purpose of determining I^{131} in milk, it was necessary to ensure that the proteins obtained did not contain other γ-emitting radionuclides, generally observed in milk, such as K^{40}, Cs^{137}, or Ba^{140} + La^{140}. Murthy *et al.* (11) had previously shown that the alkaline earths Ca, Sr, and Ba were quantitatively recovered in the trichloroacetic acid (TCA) filtrate when milk was precipitated with TCA and the remaining proteins did not contain any potassium. To confirm the absence of γ-emitting radionuclides, proteins from several milk samples were prepared and counted for gross γ-activity, and the results were compared with those of distilled water counted under identical conditions. The data presented in Table 3 show no significant differences between the two results,

TABLE 3

Gross γ-activity of milk proteins and water

Sample	Gross γ-activity (counts/min)	
	Milk proteins	Distilled water
1	323	324
2	323	326
3	336	333
4	327	330
5	320	325
6	332	330
Av.	327	328

indicating that the proteins are free of γ-emitting radionuclides except the I^{131} which, if present, was preferentially bound with protein in the presence of formaldehyde.

Although this method is adequate for the determination of I^{131} in milk under the experimental conditions studied, it may not be satisfactory in the presence of short half-lived radioiodines. The samples obtained in the vicinity of test sites immediately after weapons testing may yield higher values for I^{131} than expected. In such cases the method would yield total radioactive iodine and may be used as a screening mechanism. In a monitoring program where the samples are obtained from different parts of the country, the short half-lived radioiodines would not be expected to present any problem. Since the available data on thyroid activity have been shown to be due to I^{131} (15), it may be assumed that activity found in milk produced in areas other than the vicinity of the test site is essentially I^{131}. Therefore, the method of gross γ-counting of milk proteins should adequately measure the concentration of I^{131}.

Reproducibility of the method. Protein samples were prepared as described from 500-ml. portions of the same milk which previously had been treated with 0.5 ml. of 40% formaldehyde. The gross γ-activity of the samples was determined and compared with the total I^{131} value obtained on an aliquot of milk sample by gamma-ray spectroscopy (2). The results presented in Table 4 show

TABLE 4
Reproducibility of iodine[131] determination

Sample	Total I^{131a}	Protein-bound I^{131b}	Recovery
	(μμc.)	*(μμc.)*	*(%)*
1	1,010	1,033	102
2	1,010	985	98
3	1,010	1,004	99
4	1,010	1,012	100
5	1,010	1,003	99
6	1,010	984	98
			Av. 99 ± 2%

[a] By gamma spectroscopy (2).
[b] By gross γ-counting.

that this method of I^{131} determination is reproducible and that recovery of I^{131} is 98 ± 2%.

Special problems in counting gross γ-activity. To convert the observed counting rates to absolute activity (disintegrations per minute), the efficiency of the counter must be determined. Counter efficiency is a function of geometry and should be maintained if samples are to be compared or if sample size varies. In the latter case the counter should be calibrated for each sample size. The efficiency of the gross γ-counter employed in the present investigation was determined by using tracer I^{131} obtained from Oak Ridge. An aliquot of the tracer containing a known activity was transferred to an 8-oz. polyethylene bottle calibrated to 10-, 50-, 100-, 150-, 200-, and 250-ml. levels. The I^{131} solution was counted in succession after dilution to each mark with distilled water. Counter efficiency was calculated by relating the observed to the absolute activity. The results are presented in Figure 2.

Treatment of data. To convert counting rates of sample to absolute units (μμc.), the observed counts per minute are divided by 2.22 ER, where E is the fractional efficiency of the counter, R is the fractional recovery, and 2.22 disintegrations per minute represents 1 μμc.

Since I^{131} has a half-life of 8.05 days, the counting rates must be corrected for decay from time of sample collection to time of counting by the relationship:

$$A = A_o e^{-\lambda t}$$

where A is the observed activity, A_o is the activity at the time of collection, t is the interval between collection and counting times (in days), and λ is the decay constant equal to $0.693/t_{1/2}$, with $t_{1/2}$ the half-life of I^{131}. The corrected counting rates are converted to absolute units as indicated above.

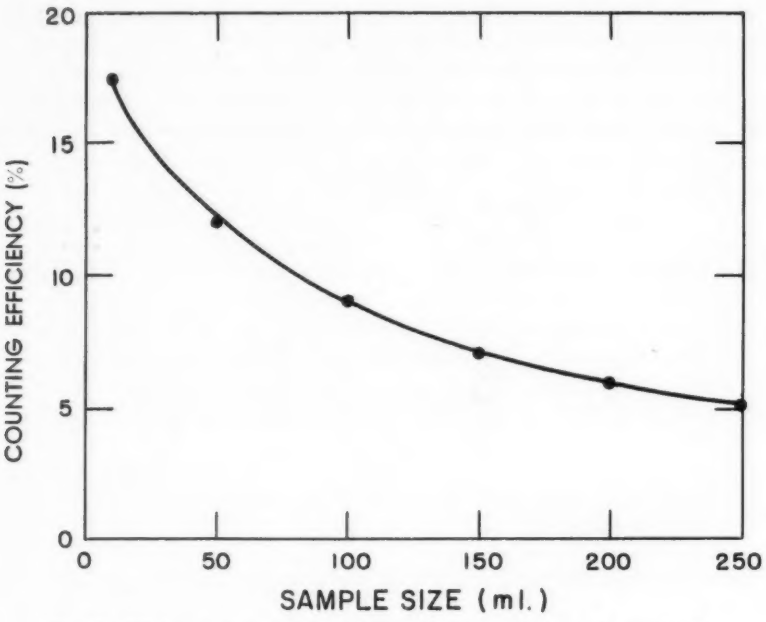

FIG. 2. Efficiency of gross γ-counting of I^{131} as a function of sample size.

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A POTENTIOMETRIC METHOD FOR THE DETERMINATION OF CHLORIDE IN MILK[1]

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SUMMARY

When chlorides in milk are titrated directly with silver, the results are too high because of interference by protein. This interference can be eliminated by titration in nitric acid solution at a pH near 2.0. A pure silver electrode can be used to detect the end point in the acid solution. This electrode may be substituted for the glass electrode of a pH meter, using a saturated calomel electrode of the asbestos fiber type as reference electrode.

If the use of nitric acid is considered undesirable, it is possible to substitute potassium aluminum sulphate for the nitric acid but much more is required. The results are almost the same as with nitric acid. If necessary, a correction should be made for chloride in the alum.

This potentiometric procedure at pH 2.0 is practically as fast as the direct chromate titration and it is believed to avoid the errors involved in other procedures which have been proposed.

The determination of the chloride content of milk is of practical importance because high values for chlorides are an index of abnormality. High values are associated with mastitis milk, with colostrum, and with milk from cows in the last stages of lactation. Recently, it has been suggested (5) that more accurate values for total solids might be obtained if lactometer readings were corrected for variation in the chloride (and lactose) content of milk.

The most simple method for the determination of chlorides is to titrate the milk directly, using potassium chromate as indicator. With practice, reasonably reproducible results can be obtained, though the results are higher than chloride determinations made after dry ashing of the milk. Hammer and Bailey (4) reported that the difference in the two methods averaged 0.025% chloride, and they attributed the error principally to the casein. If this is true, the difference should vary with the protein content of the milk. Sharp and Struble (6) studied the direct titration with chromate indicator. When the titration mixture contained less than 1% of K_2CrO_4, variations in the amount of chromate indicator had a marked effect upon the apparent chloride content of the milk. They recommended the use of 1% of chromate in the sample. Smaller amounts of indicator resulted in higher titration values. Large amounts of indicator have another advantage. Silver chromate is soluble in acid solution and a distinct end point is not obtained in sour milk unless an excess of chromate is added. Chromic acid is a weak acid and potassium chromate acts as an alkaline buffer raising the pH of the titration mixture.

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[1] Early papers on argentometric methods for chloride were reviewed by George P. Sanders, The Determination of Chloride in Milk, J. Dairy Sci., 22: 841. 1939.

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By ashing the milk, we may eliminate interference by casein at the expense of simplicity of procedure and time required. However, we introduce a new source of error. Analysts usually avoid heating the ashing furnace above 500° C., but the ash itself is heated much hotter than the furnace temperature by the oxidation of the carbon mixed with the ash constituents. It is difficult to estimate how much chloride may be lost. Some analysts have used nitric acid to speed up the oxidation. This practice seems unwise because HCl is more volatile than HNO_3 and because free chlorine may be formed.

Some have advocated a wet ashing procedure. Davies (2) recommended a wet ashing procedure, using potassium permanganate and concentrated nitric acid after the chloride had been bound by the addition of a measured amount of silver nitrate added in excess. A considerable volume of highly toxic and corrosive fumes is evolved. The reaction must be conducted in a hood with an adequate draft.

After the solution is cleared and decolorized by the addition of urea, the excess silver may be titrated directly with KCNS, using ferric ions as indicator. It is advisable, however, to filter out the precipitate of AgCl before titration, because KCNS can react with AgCl. Unless the precipitate is removed, this reaction causes the end point to fade and increases the volume of KCNS used. Unfortunately, filteration is time-consuming, and the precipitate adsorbs excess $AgNO_3$ which can not be removed easily by washing (3).

Two procedures have been proposed to avoid the necessity of removing the AgCl before titration with KCNS. One procedure (1) involves the addition of nitrobenzene, a liquid insoluble in water, which apparently wets the surface of the silver chloride precipitate and prevents rapid reaction with ions in the aqueous phase. The other procedure (7) involves the addition of such a large amount of ferric indicator that, because of mass action, the indicator will react with the excess of CNS^- ions before they can react with the precipitate of silver chloride.

Although several methods for the determination of chloride are available, each has its disadvantages and we might hope for a simpler and more accurate procedure. Direct titration is the most simple method, but high results are obtained because of the protein present. It seemed reasonable that protein would not interfere if the titration were performed in a strongly acid solution but, in acid solution, it would be necessary to use some indicator other than chromate. It seemed likely that the potential of a silver electrode would serve this purpose. The idea seemed practical because most laboratories are now equipped with pH meters which can be used to measure the electrode potential.

EXPERIMENTAL PROCEDURE

As a result of experience extending over a period of more than 10 yr., the following procedure is suggested. Pipette 10 ml. of milk into a 100-ml. beaker. We find that this sample weighs approximately 10.19 g. (A pipette calibrated to deliver 10.00 ml. of water does not deliver that volume of milk.) Add 20 ml. of dilute nitric acid (6.0 ml. of concentrated nitric diluted to one liter). This

mixture of milk and acid has a pH of approximately 2. At this pH, the casein is finely dispersed and does not form large clots which might trap chlorides. Place a small plastic-coated magnetic stirring bar in the beaker, insert the electrodes, and titrate with silver nitrate to a potential of +250 mv. with respect to a saturated calomel electrode.

The negative electrode is an ordinary Beckman calomel electrode with an asbestos fiber tip. The amount of chloride diffusing through the fiber causes no measurable error in the titration. The positive electrode consists of a short spiral of pure silver wire mounted in an insulated support. Do not use sterling silver, because it is an alloy containing copper. The silver wire is connected to the potentiometer by means of a lead taken from a discarded glass electrode. This provides the special connector needed for insertion in the Beckman pH meter. Alternatively, a silver electrode may be purchased from Beckman Instruments, Inc.

The electrode will become sluggish if fat or protein films are allowed to accumulate upon the surface. Whenever this happens, the electrode must be cleaned. Our electrode is mounted in a rubber stopper which can be fitted into the neck of a small bottle containing water and 100-mesh carborundum powder. The electrode can be cleaned quickly and easily by shaking it with this abrasive mixture whenever that is necessary.

The silver nitrate solution contains 4.882 g. of $AgNO_3$ per liter, 1 ml. of this solution is equivalent to 0.0100% of chloride in a 10.19-g. sample (10.00 ml). (This concentration may be adjusted to match the mean weight of sample delivered by the pipette used to measure the milk.)

The magnetic stirrer is a convenience, not a necessity. Its motion causes some vibration of the galvanometer when titrating solutions of low electrical conductivity, but the effect is negligible when nitric acid (or alum) is added.

This procedure has been compared with the dry ashing method and with the wet ashing method. The results are in close agreement. Typical results are shown in Table 1. There is reason to believe that the electrode titration results are actually more accurate than the others.

TABLE 1

A comparison of a direct titrimetric method with the thiocyanate titration method for the estimation of chlorides in cow's milk

Cow No.	Titrimetric method[a]			Thiocyanate method[b]		
	(A)	(B)	Ave.	(A)	(B)	Ave.
	(% chloride)			*(% chloride)*		
1	0.102	0.102	0.102	0.099	0.104	0.102
2	0.081	0.083	0.082	0.078	0.080	0.079
3	0.081	0.081	0.081	0.081	0.083	0.082
4	0.084	0.085	0.085	0.081	0.082	0.082

[a] An 18-g. sample of milk was delivered into a 100-ml. beaker from a Babcock milk pipette. Thirty ml. of 10% potassium alum was added; and the solution titrated with $AgNO_3$.

[b] Essentially, the Official A.O.A.C. method for cheese was followed, using an 18-g. sample of milk. However, filtering of AgCl was eliminated and 1 ml. of nitrobenzene added before titration with KSCN.

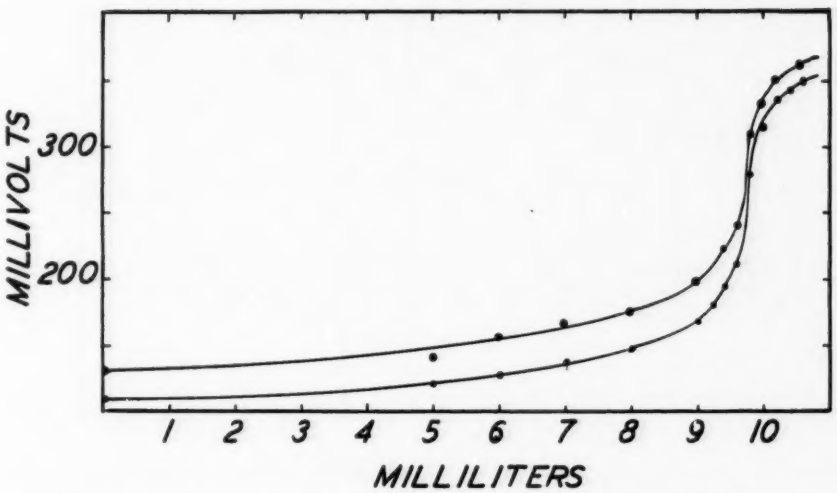

FIG. 1. Potentiometric titration curves of a solution of KCl with added nitric acid (lower line) and without. Potential of a silver electrode with respect to a saturated calomel electrode versus the volume of silver solution added.

An examination of the titration curves obtained under different conditions throws light upon some of the factors involved. Figure 1 shows the curves obtained when titrating a solution of pure KCl with, and without, the addition of nitric acid. Both curves show a sharp rise in potential at the end point. (Based on weight of KCl used, the end point should be at 9.75 ml.) According to theory, the curves should be symmetrical about the end point. From this fact, we note that both curves agree as to the volume of $AgNO_3$ required for the titration, but they differ slightly in potential at the end point. This may be due to the effect of nitric acid upon the solubility of the silver nitrate, or to the effect of ionic strength upon the activity of the silver ions. We can ignore this effect so long as we use the center of symmetry to identify the end point.

Figure 2 consists of two parts. Each represents the titration of the same quantity of KCl titrated in Figure 1. The sample titrated in 2A contained 1.0% of K_2CrO_4 as indicator, the concentration recommended by Sharp and Struble. Precipitation of excess silver as silver chromate checked the rise in potential at approximately 255 mv. This represents the end point with chromate indicator. The indicated volume of silver nitrate solution is in close agreement with the titration of Figure 1. The sample titrated in 2B contained 200 mg. of K_2HPO_4 in addition to the KCl. The pH of this solution, after titration with $AgNO_3$, was 8.5. At this pH, the phosphate interfered with the titration at the end point and more silver was required. The effect of phosphate should be less in more acid solutions and may be of little importance in the ordinary chromate titration. The pH is near 7.5 when 1% of chromate indicator is used.

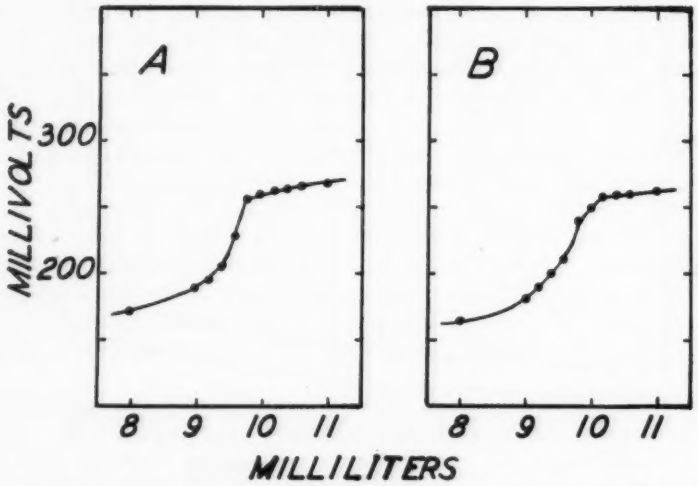

FIG. 2. The effect of K_2CrO_4 (A) and of K_2HPO_4 (B) upon the form of the titration curve. Compare with Figure 1.

Figure 3 shows the titration of diluted milk with, and without, 1% of added potassium chromate. The curve obtained after the addition of chromate is shifted downward. This effect is similar to that produced by nitric acid. Both curves have an abnormal form indicating that some interfering substance is binding silver ions and thus preventing a sharp rise in potential at the end point. When titrating the solution containing chromate, a brown color appeared at a potential of 255 mv. There is a break in the slope of the line at this point due to the precipitation of silver chromate. If we consider this break as the end point, the titration required 12.85 ml. Only 12.35 ml. was required in the absence of chromate to reach this same potential. Part of the difference may be attributed

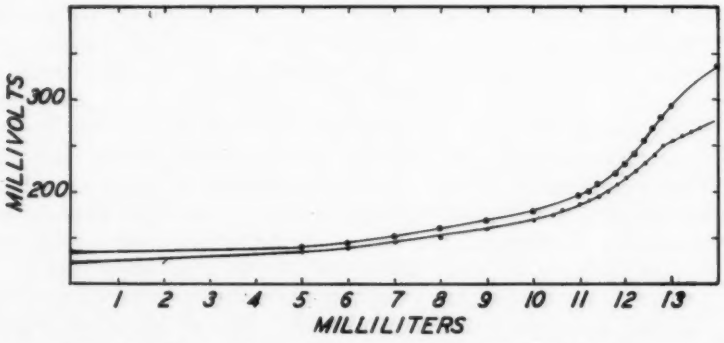

FIG. 3. Potentiometric titration curves of chloride in milk with added chromate (lower line) and without. Compare form of the curves with those in Figure 1.

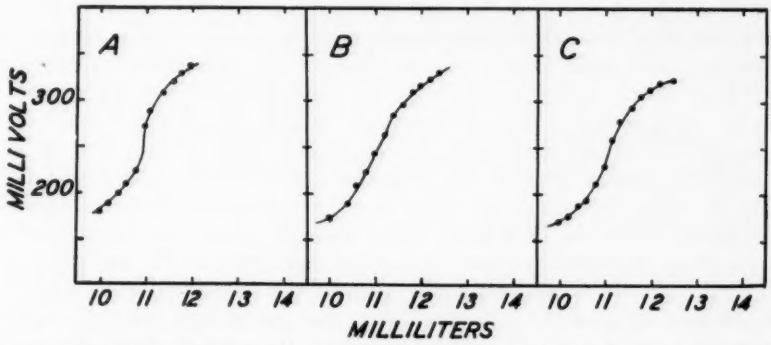

FIG. 4. The effect of nitric acid, pH 2.0 (A), and of two levels of alum upon the form of the titration curves for chloride in milk. Compare with Figure 3 and with Figure 1. B contains two volumes of 5% alum solution. C contains two volumes 10% alum solution.

to the fact that chromate raises the pH and increases the amount of error caused by protein.

Figure 4 shows the end point region of titration curves of the same milk under other conditions. Part A indicates what happened when the milk was diluted with 20 ml. of dilute nitric acid. The pH at the end point was 2.0. This curve has a normal form, indicating that there is little interference in the neighborhood of the end point. The curvature reverses near 250 mv, the same value found when titrating acidified KCl. The volume of $AgNO_3$ required is approximately 10.90 ml. In comparison, the titration with chromate required 12.85 ml. (Figure 3). Evidently, the acid has prevented interference by substances equivalent to 0.0195 of chloride ion.

Experiments with milk dialyzed for several days at low temperature indicated that the protein was responsible for most of this error. The protein error could be eliminated by adding enough nitric acid to lower the pH below 3.0. The results of a typical experiment with dialyzed milk are shown in Table 2. In other experiments, it was found that interference by protein increases with pH up to 9.25, the highest value tested.

Although dialyzed milk proteins required significant amounts of silver when titrated at 7.5 (the approximate pH of the titration with chromate indicator), the amount required was less than expected. The reason for this is not known. Perhaps the phosphate, in combination with other diffusible ions, causes more error than was supposed.

TABLE 2

Apparent chloride content of a dialyzed milk titrated at different pH values

pH	% Cl^-	pH	% Cl^-
7.18	0.0112	3.07	0.0000
6.07	.0078	2.76	.0000
5.50	.0056	2.36	.0000
4.48	.0014	2.13	.0000
3.57	.0003	1.70	.0000

This information suggested that the interfering effect of the protein might be controlled by the addition of a positive polyvalent ion. It was thought that potassium alum might be effective and that its use might be advantageous in some laboratories. It is readily available, and it is safer than nitric acid. It does not stain, and it is far less corrosive. Experiments showed that it could be used, but relatively large amounts are required. This is illustrated by Parts B and C of Figure 4. Part B shows data for the same milk diluted with 20 ml. of 5% w/v hydrated potassium alum. Part C shows similar data when the milk was diluted with 20 ml. of 10% w/v alum solution. With the higher concentration of alum, the curve is almost normal in shape, though the vertical part is shortened. (Compare with 4A.) When the lower concentration of alum was used, the rise in potential at the end point was less abrupt, but the volume of silver nitrate required in this case was almost the same as when using nitric acid or the higher concentration of alum. With other samples of milk significant errors were observed when using the lower concentration of alum.

The alum method may be useful if the use of nitric acid is considered undesirable. It yields almost but not quite the same values as the nitric acid procedure. The values are about 0.0012% higher with alum than with nitric acid. This is approximately 1/20th of the error of the chromate titration. This error may be explained in part by the fact that the pH of the alum-milk mixture is too high, about 3.5 instead of 2.0.

The use of alum may be criticized because the casein is coagulated and chloride may be trapped in the curd. If the milk is added to the alum solution, a more finely divided curd is obtained. We have not observed any trouble from the curd when using a magnetic stirrer to give vigorous agitation. The need for cleaning of the electrode has been mentioned. The need for cleaning is revealed by the sluggish movement of the galvanometer. It should come to rest almost instantly after silver nitrate is added, provided the stirring is adequate.

As a test of the precision, or reproducibility, of the method in unskilled hands, a sample of milk was divided into two portions. Potassium chloride equivalent to 0.0142% of chloride was added to one portion. Eight students (graduate and undergraduate) were asked to determine chlorides in these samples, using nitric acid and a silver electrode. Each made only one titration of each sample using a burette graduated in tenths milliliter. Only one had ever used the method before. None knew the results reported by the others. Their results are summarized in Table 3.

TABLE 3

A comparison of results reported by eight different individuals on the same samples

	Per cent of chloride		
	Original sample	Same + 0.0142% chloride	Difference
Maximum	0.1075	0.1215	0.0145
Minimum	0.1060	0.1203	0.0134
Mean	0.1068	0.1208	0.0139

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A STUDY OF THE RIPENING OF DARIWORLD AND CHEDDAR CHEESE WITH SPECIAL EMPHASIS ON PROTEOLYSIS[1]

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SUMMARY

Smooth, waxy consistency and desirable processing characteristics develop more rapidly in Dariworld than in Cheddar cheese. Measurements of soluble nitrogen and formol nitrogen were made of both varieties during 5 mo. of ripening to learn if rates of proteolysis might explain these differences in consistency. Observations were made at five different stages of ripening on two lots of each variety, one lot of which was normal and the other abnormally high in pH. Rates of proteolysis were essentially alike in the normal lots of both varieties and in the Cheddar with high pH. The sweet Dariworld which, unlike the sweet Cheddar, retained its high pH during ripening, showed the most rapid and extensive protein decomposition. Both of the sweet lots, however, were hard and woody in consistency and did not improve as amounts of soluble nitrogen and formol nitrogen increased during ripening. Acidity developed in making the sweet modifications evidently was insufficient to effect normal partition of minerals between whey and curd, and the resulting abnormal composition of curd apparently caused the defects in body. Rapid loss of curdiness in the ripening of normal Dariworld was not caused by unusually fast protein breakdown. Physico-chemical changes caused by the prolonged exposure of the curd to sodium chloride brine during the making operation of this cheese and the relatively high moisture content evidently account for its characteristic consistency.

Dariworld, a soft ripened cheese, develops a smooth, waxy body approximately 2 wk. after making. It has a mild flavor which is stable during storage. The rapid development of waxy consistency and the mild flavor makes the cheese useful for processing. It has been used when relatively young as a base for pasteurized process cheese spreads (10, 13). The limitations of using fresh Cheddar cheese for processing have been recognized (2, 15, 21). Contrasts in rates of loss of curdiness and in processing characteristics of Dariworld and Cheddar cheese in early stages of curing suggested differences in ripening which might be related to extent of protein decomposition. The experiments reported in this paper were made to investigate this possibility.

EXPERIMENTAL METHODS

The following procedures were repeated on separate days to make five trials or replicates.

Two vats of Dariworld and two of Cheddar were manufactured on the same day from the same pasteurized milk obtained from the mixed milk supply of the dairy manufacturing laboratory of the University of Wisconsin. One vat

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of each variety was made in the normal way (10, 24), whereas the second was made to produce a high pH in the finished cheese. The high pH was attained by reducing the amount of starter, limiting acid development before renneting, delaying acid development, and firming the curd by heating it in whey to 104–106° F. in the low-acid Cheddar, and to only 95° F. in the low-acid Dariworld. Each vat lot of cheese was divided into 5-lb. units, wrapped in Parakote, and cured at 50° F. At two days of age each lot was analyzed for moisture, fat, and pH. At 10, 30, 60, 90, and 150 days of age, each cheese was scored for body, texture, flavor, and total score, and analyzed for proteolytic changes by measuring soluble nitrogen and formol nitrogen.

Proteolysis. The rate of protein breakdown during ripening was followed by periodic measurements of nitrogen and formol nitrogen soluble at pH 4.4 ± 0.05 in a sodium citrate–HCl extract of the cheese. This extract was prepared essentially by Mogensen's method (12, 22). The soluble nitrogen in this extract measured by Kjeldahl analysis indicates extent of proteolysis. The formol nitrogen which is calculated from the formol titration of the extract reflects the amount of nitrogen in free amino groups of the soluble products of proteolysis present in the extract. The formol titration thus indicates the progress of protein breakdown to small peptides and amino acids.

Total nitrogen was measured by taking a 1-ml. aliquot of the nonacidified, milky, sodium citrate solution and using the semimicro-Kjeldahl procedure (9).

Formol titration was made on the acidified and filtered sodium citrate–HCl extract of the cheese. To 25 ml. of the extract were added five drops of a 2% solution of phenolphthalein in ethanol and 2.6 ml. of 1 *N* NaOH solution, followed by careful titration with 0.1 *N* NaOH to match a color standard. This color standard consisted of 25 ml. freshly prepared borate buffer, pH 8.3, plus five drops of the 2% phenolphthalein solution. After this adjustment of the cheese extract to pH 8.3, 10 ml. of 30-40% formaldehyde was added and the solution titrated again with 0.1 NaOH until the color again matched the color standard. This titration in milliliters of 0.1 *N* NaOH, minus the milliliters of 0.1 *N* NaOH required to titrate 10 ml. of formaldehyde to the color standard, was recorded as the formol titration value of the cheese. Since one equivalent of normal alkali corresponds to one equivalent of amino groups, the formol titration was used to calculate formol nitrogen, which was expressed as per cent of the total nitrogen of the cheese as suggested by Mogensen (12).

Moisture. Cheese was analyzed for moisture by drying 3-5 g. in 50-ml. beakers at 110° C. for 16 hr. in a forced draft oven (24).

Cheese pH. The pH of cheese was measured with a saturated calomel half-cell, gold electrode, and a Leeds and Northrup portable potentiometer (Catalog No. 7655) (17, 24).

Statistical methods. The statistical design used in analyzing the data from the four vat lots of cheese was a factorial of the split-plot type (4, 19, 20). The basic design was a randomized complete block. The two varieties, Dariworld and Cheddar, with each at the normal and high pH levels, constituted four main treatments, while the ages at which the cheeses were scored and

analyzed represented five sub-treatments. For convenience in discussing them, the four main treatments will be referred to as modifications.

Cheese scores. The cheeses were evaluated by a panel of three to five experienced judges, who used a scale of 1 to 6 for each characteristic of the cheese. Scores of 1 were excellent, 2—desirable, 3—satisfactory, 4—objectionable, 5—very objectionable, and 6—unsalable. Total scores were made, using the same scale to summarize the judge's general opinion of each sample.

RESULTS

Quality of cheese. Averages of flavor, body, and total scores of the cheese modifications throughout ripening are shown in Table 1. Analysis of variance

TABLE 1

Quality of Dariworld and Cheddar cheese modifications
(Values are averages of scores of cheese at 10, 30, 60, 90, and 150 days of age for each of five trials)

Cheese modification	Flavor scores	Body scores	Total scores
Normal Dariworld	2.54	1.52	2.18
Sweet Dariworld	3.20	3.20	3.34
Normal Cheddar	2.40	1.70	2.04
Sweet Cheddar	2.95	2.61	2.82

showed highly significant differences (1% level) in flavor, body, and total scores between the cheese modifications. Only the flavor scores within the cheese modifications showed significant differences (5% level) at the several ages. Flavors of all modifications did not react the same during aging.

By Duncan's multiple-range test (8), Table 2 shows comparisons of mean scores of the cheese modifications made in the five trials at every scoring period. Total scores (Table 1) of normal Dariworld and normal Cheddar, which averaged 2.18 and 2.04, respectively, in five scorings were essentially alike throughout aging; sweet Dariworld and sweet Cheddar, which averaged 3.34 and 2.82, respectively, in five scorings were inferior to the normal cheese after ten days of age. At 60 and 90 days total scores of sweet Dariworld were 3.66 and 3.84, respectively, and were inferior to all other modifications. The body scores of the four modifications showed significant differences beginning at ten days of age. The body scores of sweet Cheddar and sweet Dariworld were always inferior to the normal cheese at 30 days and later. After 60 days the sweet Dariworld, with scores of 3.36 at 90 days and 3.52 at 150 days, was inferior to the other three modifications. Flavor scores were not significantly different statistically, according to Duncan's test (8), until after 90 days, when the sweet Dariworld with a score of 3.90 was inferior to all other modifications.

The faults of the sweet modifications were in general more openness of texture and sweet, unnatural flavors. These faults were exaggerated in sweet Dariworld which developed large sweet-holes and Swiss-like flavor.

Moisture in cheese. The percentages of moisture in the modifications were quite typical. Average values and standard deviations for the five trials were:

TABLE 2

Ranking of total, body, and flavor scores of the Cheddar and Dariworld cheese modifications in order of acceptability at the various ages

Age of cheese (days)	First	Second	Third	Fourth
		Rank[a] of mean total scores		
10	2.22 (CN)	2.38 (DN)	2.98 (CS)	3.02 (DS)
30	1.98 (DN)	2.08 (CN)	2.76 (CS)	3.02 (DS)
60	1.82 (CN)	2.12 (DN)	2.80 (CS)	3.08 (DS)
90	2.04 (CN)	2.14 (DN)	2.66 (CS)	3.66 (DS)
150	2.04 (CN)	2.26 (DN)	2.90 (CS)	3.84 (DS)
		Rank of mean body scores		
10	1.82 (DN)	1.98 (CN)	2.66 (CS)	3.18 (DS)
30	1.32 (DN)	1.40 (CN)	2.66 (DS)	2.70 (CS)
60	1.42 (DN)	1.54 (CN)	2.64 (CS)	3.30 (DS)
90	1.54 (DN)	1.58 (CN)	2.36 (CS)	3.36 (DS)
150	1.50 (DN)	1.90 (CN)	2.70 (CS)	3.52 (DS)
		Rank of mean flavor scores		
10	2.40 (CN)	2.88 (CS)	2.90 (DN)	2.96 (DS)
30	2.20 (DN)	2.36 (CN)	3.22 (DS)	3.26 (CS)
60	2.20 (CN)	2.34 (DN)	2.52 (DS)	2.80 (CS)
90	2.62 (CN)	2.84 (DN)	2.90 (CS)	3.38 (DS)
150	2.38 (CN)	2.40 (DN)	2.90 (CS)	3.90 (DS)

[a] Any means not underscored by the same line are significantly different at the 5% level (27). Letters in parentheses identify the modification associated with each mean: DN = Normal Dariworld; DS = Sweet Dariworld; CN = Normal Cheddar; CS = Sweet Cheddar.

TABLE 3

Soluble nitrogen as per cent of total nitrogen in the Cheddar and Dariworld modifications at 10, 30, 60, 90, and 150 days of age

Age of cheese	Mean[a]	95% Confidence limits	Mean[a]	95% Confidence limits
(days)		*(%)*		
	Normal Cheddar		Normal Dariworld	
10	8.80	8.04– 9.56	9.60	8.61–10.59
30	12.75	12.38–13.12	13.35	12.43–14.27
60	17.12	16.02–18.22	17.81	15.84–19.78
90	20.57	19.63–21.51	21.26	19.40–23.12
150	26.11	25.09–27.13	28.23	26.60–29.86
	Sweet Cheddar		Sweet Dariworld	
10	9.71	8.45–10.97	8.47	7.78– 9.16
30	12.67	11.49–13.85	12.80	11.89–13.71
60	17.72	15.99–19.45	18.77	16.22–21.32
90	21.07	18.47–23.67	23.26	21.05–26.27
150	26.21	24.06–28.36	31.47	27.80–35.14

[a] Each mean is the average of five trials.

normal Dariworld—43.97 ± 1.84, sweet Dariworld—44.34 ± 0.96, normal Cheddar—39.06 ± 0.97, and sweet Cheddar—42.04 ± 1.30.

Acidity of cheese. The changes in pH of the cheese modifications from salting to 150 days are shown in Figure 1. The pH of the normal lots of Dariworld and Cheddar decreased during the first ten days to approximately 5.1 and then gradually increased to the end of the period of observation. The pH of Dariworld increased slightly more rapidly to nearly 5.3 pH. The trends were normal for both varieties. The differences in pH of approximately 0.5 to 0.8 at ten days of age between the normal and sweet modifications were maintained later in curing only by the sweet Dariworld. The sweet Cheddar lots decreased slowly in pH after making until, after 60 days of curing, they were only 0.1 to 0.2 pH unit higher than the pH of normal Cheddar and normal Dariworld.

Proteolysis. Averages of soluble nitrogen as per cent of total nitrogen in the cheese are shown in Table 3, with 95% confidence limits for all modifica-

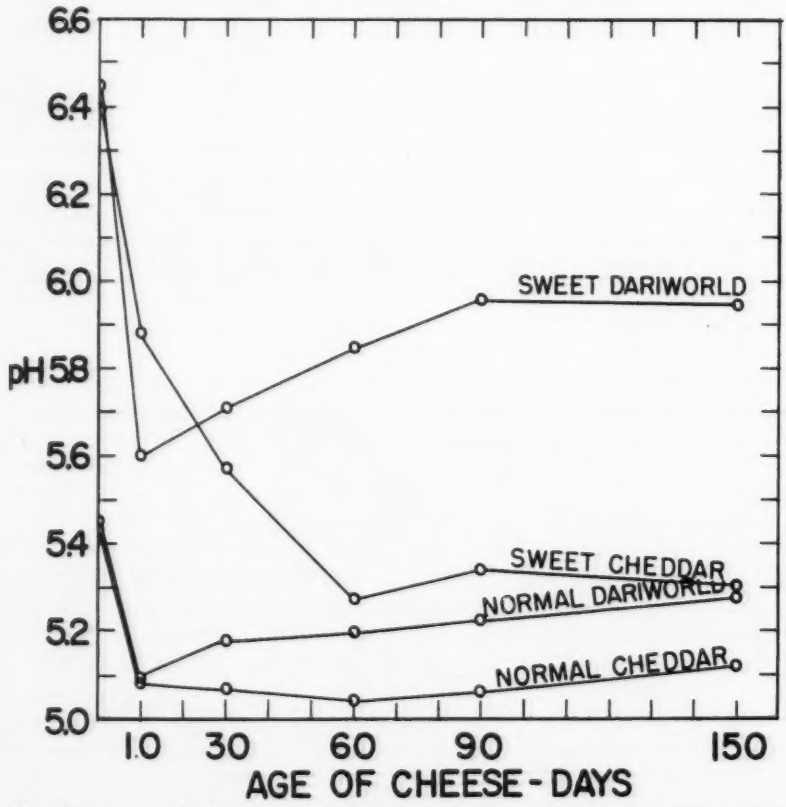

FIG. 1. Changes in pH during ripening of Dariworld and Cheddar cheese, and their sweet modifications.

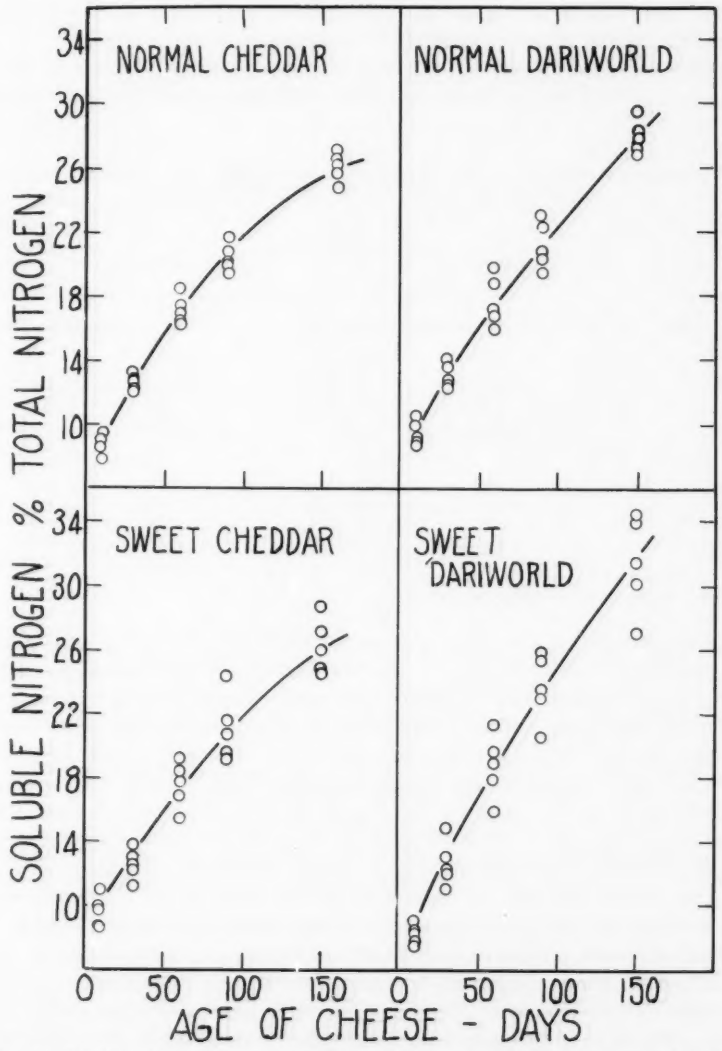

FIG. 2. Soluble nitrogen as per cent of total nitrogen developed in normal and sweet modifications of Cheddar and Dariworld cheese during 5 mo. of ripening.

tions at every period of observation during 5 mo. of ripening. The trends of these observations are illustrated in Figure 2. Increases in soluble nitrogen as per cent of total nitrogen in all modifications of Cheddar and Dariworld cheese were statistically alike by Duncan's multiple range test (8) at 10, 30, and 60 days of curing. After 60 days the sweet Dariworld contained approximately

2 to 3% more soluble nitrogen than the normal Dariworld and 3 to 5% more than either normal or sweet Cheddar at comparable ages.

Formol nitrogen as per cent of total nitrogen increased during ripening of all cheese. Table 4 shows means and 95% confidence limits for each modification at every stage of the 5-mo. ripening period. The trends of these observations are illustrated in Figure 3. The variability of observations among replicates of each modification increased with age. Duncan's test (8) showed no statistically significant differences between modifications for the first 30 days of ripening. At 60, 90, and 150 days, the sweet Dariworld was significantly higher in formol nitrogen than all other modifications. It was most nearly like normal Cheddar in formol nitrogen content, but exceeded that of normal Cheddar by approximately 1, 2, and 4% at 60, 90, and 150 days, respectively. The similarity of formol nitrogen content in normal and sweet Cheddar cheese indicates that initial pH had no significant effect, directly or indirectly, on the breakdown of protein to peptides as it apparently did in Dariworld. It is important to recall, however, that the pH of sweet Cheddar (Figure 1) gradually decreased during ripening so that it approached that of normal Cheddar and normal Dariworld after 60 days. This trend is a marked contrast to the high (> 5.6) level of pH maintained throughout ripening by sweet Dariworld.

TABLE 4

Formol nitrogen as per cent of total nitrogen of the Cheddar and Dariworld modifications at ages 10, 30, 60, 90, and 150 days during ripening

Age of cheese	Mean[a]	95% Confidence limits	Mean[a]	95% Confidence limits
(days)		*(%)*		
	Normal Cheddar		Normal Dariworld	
10	0.70	0.34–1.06	0.58	0.40– 0.76
30	1.81	1.48–2.24	1.57	1.06– 2.08
60	3.17	2.40–3.94	2.74	1.71– 3.77
90	4.40	3.40–5.40	3.79	2.51– 5.07
150	6.70	5.42–7.98	6.24	4.35– 8.13
	Sweet Cheddar		Sweet Dariworld	
10	0.60	0.36–0.84	0.57	0.50– 0.64
30	1.38	0.82–1.80	2.01	1.62– 2.40
60	2.33	1.66–3.00	4.20	3.64– 4.76
90	3.20	2.26–4.14	6.48	5.23– 7.73
150	5.23	3.79–6.67	11.25	9.62–12.88

[a] Each mean is the average of five trials.

DISCUSSION

The more rapid disappearance of curdiness from Dariworld than from Cheddar might be assumed to be attributed to differences in rate and extent of proteolysis. The higher moisture content of Dariworld should accelerate proteolysis, although the heating of the Dariworld curd in brine to approximately 120° F. might be expected to inhibit or delay bacterial and enzyme actions. The Dariworld and Cheddar in each replicate trial were made from

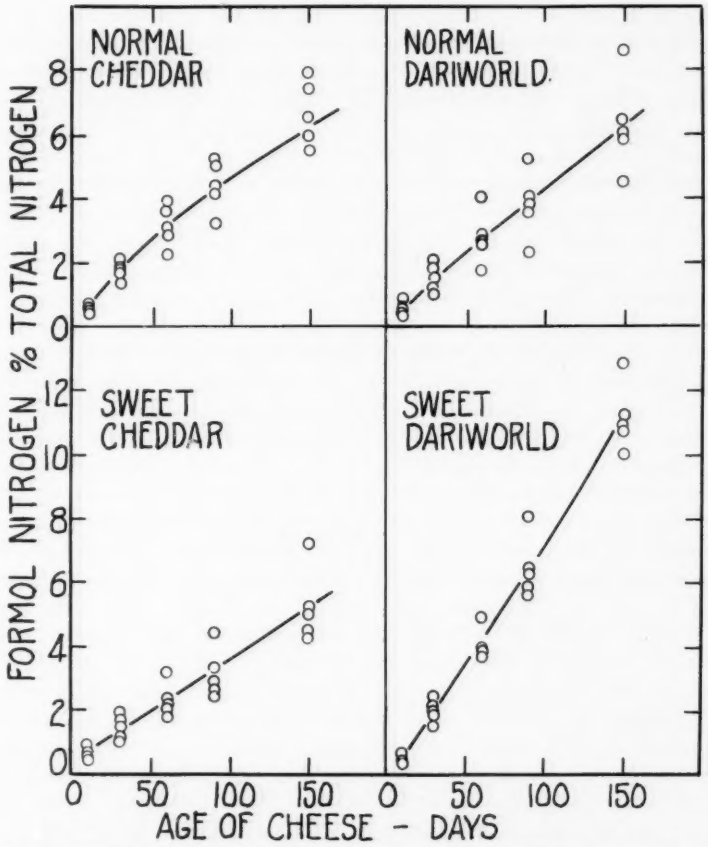

FIG. 3. Formol nitrogen as per cent of total nitrogen developed in normal and sweet modifications of Cheddar and Dariworld cheese during 5 mo. of ripening.

identical milk and starter, and the cheeses were ripened in the same curing room so that variations in proteolytic effects from these sources in the four modifications were minimized. Differences in rates of proteolysis were limited in this way to the effects of methods of manufacturing.

The analytical data unexpectedly showed no statistically significant differences in the rates of production of soluble and formol nitrogen during the 5 mo. of ripening of the normal Dariworld and normal Cheddar. The patterns of proteolysis in these two types seemed to be essentially alike. Among the four modifications only the sweet Dariworld differed in rate of proteolysis from the other three modifications, and then only in the last months of the 5-mo. curing period.

It seems apparent that the loss of curdiness and the rapid development of smooth, waxy consistency of Dariworld are not the results of rapid protein

breakdown. It now seems more probable that the characteristic consistency of Dariworld may be the result of physico-chemical changes induced by the prolonged exposure of the curd to brine and the final heating of the curd in the 5% sodium chloride solution. Irvine (10) suggested that a partial interchange of sodium for calcium in the protein might occur during the brine treatments. The resulting sodium-calcium paracaseinate complex, he believed, should retain a relatively high level of water of hydration and so be responsible for the body characteristics of the Dariworld which frequently contains 7 to 10% more moisture than Cheddar. In the experiments reported in this paper the differences in moisture between these two varieties approximated only 5%, which makes the differences in rate of body changes even more striking.

The rate of proteolysis of the sweet Cheddar modification did not differ significantly from that of the normal Cheddar. This was attributed to the fact that the difference in pH between these two modifications tended to disappear during ripening. The decrease in pH of sweet Cheddar was probably caused by the unusually large amounts of lactose remaining in the fresh sweet cheese.

The sweet Dariworld exhibited more rapid proteolysis than the other modifications, especially in the later periods of observation. The total amounts of formol nitrogen eventually developed were approximately double the values of the other modifications. Formol nitrogen indicates the presence of protein degradation products of small molecular weight. Rennet action can decompose the protein only to caseoses and peptones (1, 5, 18, 23). The high levels of formol nitrogen in the sweet Dariworld were probably developed by proteases of bacterial origin (16) rather than by rennin or by proteolytic enzymes from the original milk. Bacterial endoenzymes have been demonstrated in cheese by several investigators (3, 6, 11, 14). Defective changes in flavor and gas development in the sweet Dariworld coincided with development of the larger amounts of formol nitrogen and suggest the significance of bacterial action in these phases of the ripening process. These defects in body and flavor of sweet Dariworld were always more evident than in the sweet Cheddar modification which maintained high pH for only the first 6 wk. of curing.

Both sweet modifications of Dariworld and Cheddar were hard and woody in consistency. These characteristics persisted during ripening, even though extensive proteolysis occurred. The acid developed in making these modifications was insufficient to effect normal partition of minerals between curd and whey. In these sweet modifications more minerals, particularly calcium, may have been retained in the water-free solids (17). This retention of minerals alone, or in conjunction with other factors related to the lack of acid development in the sweet modifications, emphasizes the fact that proteolysis is not the only factor concerned in the development of the normal, soft consistency of ripened Dariworld and Cheddar cheese.

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EFFECTS OF AGE AND DIET ON THE SECRETION OF PREGASTRIC ESTERASE IN CALVES[1, 2]

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SUMMARY

The effects of age and diet on the quantity of pregastric esterase secreted during the nursing of milk were studied in six Holstein calves. During the first 180 days of life, there was no appreciable change in enzyme secretion. Limited observations on two calves suggest that this trend continues through at least 1 yr. of age. Calves sustained on a diet of hay and grain secreted, when nursing milk, approximately the same quantity of pregastric esterase as did those animals restricted to a diet of milk. The output of enzyme during the actual consumption of hay and grain, however, appeared to be considerably less than that stimulated by the nursing process. There was no correlation between the total volume of saliva secreted and the output of pregastric esterase.

Although ruminant saliva has not been credited with a significant enzymatic role in the digestion of feed, pregastric secretions of the young calf contain a lipolytic enzyme (12, 14). The primary areas of tissue elaborating this enzyme, pregastric esterase, are believed to be the base of the tongue and the pharyngeal end of the esophagus (10). Moreover, extracts of these tissues from day-old calves are markedly higher in esterolytic activity than are those of corresponding tissues from older calves and mature cattle (10). Although the nutritional importance of pregastric esterase is unknown, the foregoing in vitro observations suggest that the secretion of this enzyme may diminish with increasing age of the animal. Thus, the work reported herein was initiated primarily to study, in vivo, the manner in which the secretion of pregastric esterase in the calf is affected by changes in age and the usual accompanying changes in diet.

EXPERIMENTAL PROCEDURES

Treatment of experimental animals. An open rumen fistula was established in each of six male Holstein calves according to the two-stage procedure described by Dougherty (4). The first stage was initiated between seven and 17 days of age, and the second stage was completed from seven to ten days later. The calves were assigned alternately, as received, to one of two dietary groups and placed in individual pens. Three of the calves received only whole milk during the experimental period, which was begun one to two days after completion of fistulation and terminated at 180 days of age. The daily allowance

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of milk, 1 lb. per 10 lb. of body weight, was fed from a nipple pail in equal portions at 8 A.M. and 5 P.M. A solution of minerals[4] was added to the milk prior to feeding. The second group of three calves was fed similarly to the first group for approximately 2 wk. after the experimental period began, this interval being referred to hereafter as the base period. Subsequently, milk consumption was reduced by one-half, and free access to a commercial starter (18% protein) and good alfalfa-grass hay was initiated. After approximately 2 wk. of dietary adjustment, the milk was withdrawn completely. Thereafter, the intake of starter was increased to a maximum of 4 lb. per day. The ad libitum feeding of hay was continued. Each calf also received daily 20 mg. of chlortetracycline per 100 lb. of body weight, and 10,000 and 1,000 U.S.P. units of vitamins A and D, respectively. Common salt was offered ad libitum.

The fistulas of the milk-fed group were open throughout the experiment. With the calves fed hay and grain, however, it was necessary to insert plugs to prevent the loss of ingesta.

Determination of pregastric esterase in sham-fed milk. Throughout this study, the secretion of pregastric esterase in all calves was determined by nipple-feeding[5] 5 lb. of reconstituted nonfat milk, collecting the mixture of milk and saliva from the cardial end of the esophagus (12), and assaying the lipolytic activity thereof. Such milk (1 lb. of nonfat milk solids per 9 lb. of water) was selected as the test medium, because it contains only a trace of fat that might be hydrolyzed between the time of collection and that of analysis. All collections were made at the time ordinarily used for the morning feeding. The frequency of sham-feeding was three times per week initially, but was reduced later to twice, and finally to once, per week. At the evening feeding prior to sampling, the calves receiving milk were fed as usual, whereas the hay-grain calves were offered a normal allowance of starter but were deprived of hay and water. Even with restricted feeding of the latter group, it was necessary to remove ingesta from their reticula before sampling could be initiated. The ingesta were replaced after sampling, and the normal dietary regimen was resumed.

Analysis of the sham-fed milk for its content of pregastric esterase was initiated within 1 to 2 hr. after collection. To conduct such assays, 5 ml. of thoroughly mixed sham-fed milk was added to 10 ml. of evaporated milk and 3 ml. of 1.0 *M* phosphate-citrate buffer, pH 6.0, which is the pH optimum of pregastric esterase under the conditions of this procedure. This mixture was incubated in a constant temperature water bath at 35° C. for 60 min., and subsequently placed in a second water bath at 85° C. for 15 min. to inactivate the esterase. A control was prepared for analysis by heating the sham-fed milk to 100° C. before adding it to the substrate.

[4] Composition of mineral solution (grams/liter): $CuSO_4 \cdot 5H_2O$, 1.20; $FeSO_4 \cdot 7H_2O$, 7.63; $CoCO_3$, 0.012; $MgSO_4 \cdot 7H_2O$, 185.44; and $MnSO_4 \cdot H_2O$, 1.88. The rate of addition was 1 ml. per pound of milk.

[5] Nurs-A-Calf feeder, supplied through the courtesy of R. H. Kuss and Company, Inc., Findlay, Ohio.

The incubation mixture was analyzed on a silicic acid column for total free fatty acids by a slight modification of the procedure outlined by Keeney (7). The primary changes involved the use of 38 by 230-mm. chromatographic tubes and the use of 10% *n*-butanol in hexane as the mobile phase. The packed columns contained 25 g. of the mixture of silicic acid and ethylene glycol reagent. Five milliliters of the incubated sample were acidified, mixed evenly with silicic acid, and added to the packed column. The fatty acids then were eluted with the foregoing mixture of *n*-butanol and hexane. Although fractionation of the effluent revealed that all of the acids from milk fat were present in the first 70 ml., 100 ml. were collected routinely to insure complete recovery. The acids were titrated under nitrogen to the end point of thymol blue with 0.01 *N* ethanolic NaOH. The indicator solution, 10 ml. of which was added to 100 ml. of effluent, was prepared by adding 40 mg. of thymol blue to 1,000 ml. of isopropanol and neutralizing with 0.1 *N* aqueous NaOH. The activity of pregastric esterase was recorded as the μM of free fatty acids present in 5 ml. of the incubation mixture.

Sham-fed milk is known to contain an appreciable number of bacteria (13). A preliminary study of the foregoing assay mixture, however, indicated that there was either very little or no bacterial formation of fatty acids from nonlipid constituents during the time allotted for incubation. Thus, the free fatty acids observed in the effluent originated almost completely from the hydrolysis of milk fat.

Determination of pregastric esterase in sham-fed hay and grain. At the end of the experiment, several observations were made on the pregastric esterase activity in sham-fed hay and grain obtained from one of the calves. After withholding feed and water for 12 hr., the ingesta from the rumen and reticulum was removed, and the reticulum was flushed with water. Approximately 2 lb. of either grain or chopped hay was fed, and the boluses were collected as they emerged from the cardial end of the esophagus. The boluses then were pressed into a Buchner funnel, and the saliva contained therein was removed by suction. The saliva was centrifuged to remove the particulate matter and subsequently was added to reconstituted nonfat milk in the ratio of one part saliva to four parts of milk. The resulting mixture, containing the equivalent of 20% saliva, was assayed for pregastric esterase according to the same procedure used for sham-fed milk.

Estimation of the dilution of sham-fed milk with saliva. In order that the correlation between the secretion of saliva and that of pregastric esterase might be studied, the dilution of sham-fed milk with saliva was estimated by a ratio technique. Total solids, measured gravimetrically, were used as the reference material. The formula used in making these calculations is as follows:

$$\% \text{ Dilution} = \frac{\text{Volume of saliva secreted}}{\text{Volume of milk fed}} \times 100$$

$$= \frac{(\% \text{ solids in unfed milk}) - (\% \text{ solids in sham-fed milk})}{(\% \text{ solids in sham-fed milk}) - (\% \text{ solids in saliva})} \times 100$$

Samples of unfed and sham-fed milk were analyzed individually for their solids content, whereas saliva was assumed to contain 1.2% solids, a value based on several samples of aspirated saliva.

RESULTS

All of the calves in this study remained in good health. Initial weights ranged from 82 to 108 lb., and those at the end of the test period, from 267 to 330 lb.

Relationship of age to the secretion of pregastric esterase. Measurements of pregastric esterase secretion by individual calves throughout the experimental period are presented graphically in Figures 1 and 2. Visual inspection

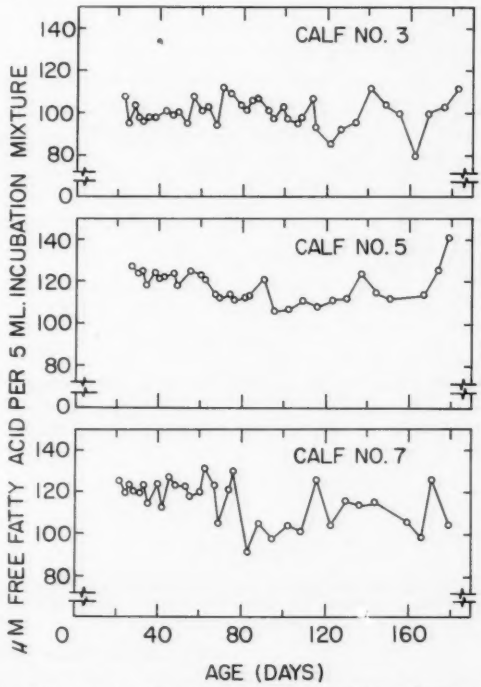

FIG. 1. Changes with age in the secretion of pregastric esterase by individual calves fed a milk diet.

of these data indicates that, with increasing age, there was no pronounced change from the initial level in the output of enzyme. This observation is substantiated by the partial correlation coefficients (11) associated with the two variables, age and enzyme activity (Table 1). Although the secretion of pregastric esterase increased significantly with age for one calf, No. 4, it decreased for another animal, No. 6, receiving the same diet. The coefficients for the other calves were not statistically significant.

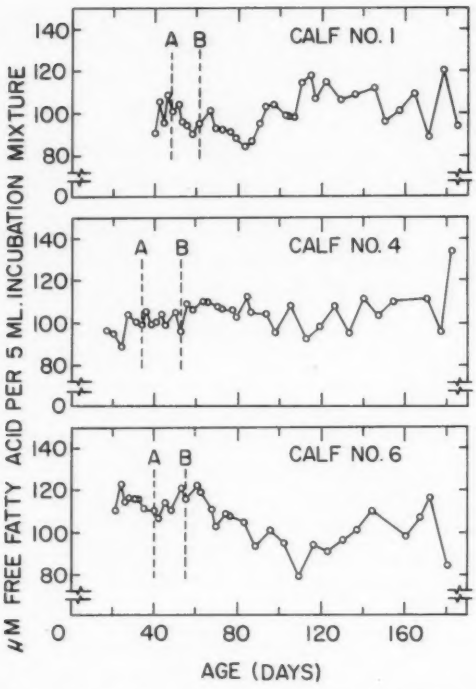

FIG. 2. Changes with age in the secretion of pregastric esterase by individual calves fed a hay-grain diet. (A = age at which hay and grain was started and milk consumption was reduced one-half. B = age at which milk was withdrawn completely from the diet.)

TABLE 1

Partial correlation coefficients between pairs of experimental variables: age of calves, activity of pregastric esterase, salivary dilution of sham-fed samples, and time required for consumption of milk

	Partial correlation coefficients[a]					
	Identification number of calves fed hay and grain			Identification number of calves fed milk		
Pairs of experimental variables	1	4	6	3	5	7
Age vs. enzyme activity, independent of nursing time and salivary dilution	.166	.449**	−.351*	−.053	−.281	−.306
Enzyme activity vs. salivary dilution, independent of age and nursing time	−.066	−.375*	−.092	−.033	−.096	−.294
Salivary dilution vs. age, independent of nursing time	.790**	.691**	.707**	.383*	.500**	.496**

[a] * and ** indicate significance of the correlation coefficients at the 5.0 and 1.0% probability levels, respectively.

The relationship between age and enzyme secretion was studied further by keeping two of the calves, one from each dietary group, until approximately 1 yr. of age. At this time, the output of pregastric esterase was measured again and found to agree closely with the level obtained at a younger age.

Relationship of diet to the secretion of pregastric esterase. The secretion patterns for calves receiving the milk diet (Figure 1) were similar to those of calves receiving hay and grain (Figure 2). Thus, the change-over from milk to dry feed did not seem to effect any marked change in the output of pregastric esterase. The general plane of secretion was slightly higher for the milk-fed calves than for the hay-grain group. This difference, which existed also during the base period when all calves received only milk, is believed to be a manifestation of individual variation rather than of diet.

At the end of the experiment, several measurements were made of pregastric esterase secretion that occurred during the ingestion of hay and grain. Only one animal, approximately 12 mo. of age, was used. Three sham-fed samples of grain and two of hay averaged 16 and 12 μM of free fatty acid, respectively, per 5 ml. of incubation mixture. During the regular test period, however, sham-fed milk collected from the same calf averaged 101 μM of free fatty acid in hydrolytic activity. This contrast in secretion of pregastric esterase is even more evident when it is considered that the sham-fed milk contained only 1 to 5% saliva (Table 2), whereas the samples from hay and grain were pre-

TABLE 2
Relationship of age and diet to the salivary dilution of sham-fed milk

Age periods	Mean salivary dilution of sham-fed milk	
	Calves fed hay and grain	Calves fed milk
(days)	*(%)*	*(%)*
16-45	1.06	1.02
46-90	0.77	1.39
91-135	2.13	1.63
136-180	4.75	2.60

pared to contain 20% saliva. Thus, these results suggest that the act of nursing stimulates the secretion of pregastric esterase to a much greater extent than does the ingestion of dry feed.

Relationship between the secretion of saliva and that of pregastric esterase. In order that the relationship between total salivary secretion and the output of pregastric esterase might be examined, partial correlation coefficients between these two variables, salivary dilution and esterase activity, were computed for each calf (Table 1). Only one of the coefficients was statistically significant at the 5% level of probability, however. Thus, the quantity of enzyme secreted during the nursing period does not seem to be related to the total volume of saliva.

Although esterase secretion was not significantly affected by either age or diet, both of these factors influenced the secretion of saliva. An increase in

the dilution of sham-fed milk was noted with increasing age of the calves fed either hay and grain or milk, but was more pronounced for those receiving hay and grain (Table 2). The increasing trend with age is illustrated further by the partial correlation coefficients between age and salivary dilution (Table 1). All of the coefficients are positive and, with the exception of one, are statistically significant at the 1% level of probability.

DISCUSSION

The observation that age is essentially without effect on the secretion of pregastric esterase does not substantiate a previous suggestion (10) that the output of this enzyme decreases as the calf becomes older. This proposal was based on the results of in vitro work with tissue extracts, some of which were prepared from calves only 8 to 36 hr. old. In the present study, however, the earliest observation of esterase secretion was made at 17 days of age. Thus, the effect of age on the secretion of this enzyme during the first 2 wk. of life is still obscure. Inasmuch as a dietary transition from milk to hay and grain failed also to affect enzyme secretion, it is assumed that the physiological changes accompanying such an alteration in diet are not directly related to the output of pregastric esterase.

Though pregastric esterase secretion seemingly is unaffected by age of the calf and the diet on which it is sustained, variability among individual calves indicates the involvement of undefined factors. Moreover, the same calf at different times frequently varied in its output of this enzyme. Although the initial level of secretion tended to persist throughout the test period, some of the calves exhibited cyclic trends in their secretion patterns. Such trends seemed too pronounced to be ascribed simply to random variation, yet they could not be correlated with any detectable changes in health or environment.

The lack of correlation between the secretion of saliva and that of pregastric esterase suggests that the glands responsible for most of the salivary flow do not secrete an appreciable quantity of this enzyme. It is possible, however, that the collection procedure, inserting a tube into the cardial end of the esophagus, may have altered the volume of saliva secreted. Kay and Ash (6) have reported that stretching the terminal end of the esophagus and stimulating physically the lips of the esophageal groove increased the flow of parotid saliva in sheep. Nevertheless, the foregoing interpretation concerning the origin of pregastric esterase is in agreement with the results of a previous study (9) on the esterolytic activity of saliva in the calf. Saliva aspirated from the base of the tongue was very high in esterase, whereas that removed from the areas where the ducts of the paired salivary glands enter the oral cavity contained almost no esterase.

No direct evidence is available on the nutritional significance of pregastric esterase. The results of assays in the present study, however, in which milk fat underwent 10 to 15% hydrolysis, suggest that this enzyme may hydrolyze more than trace amounts of dietary fat in vivo. If so, it probably is of greatest importance to the very young calf, despite the lack of a detectable age effect

on esterase secretion. Such an animal usually receives a high-fat diet, whole milk. Moreover, the nursing of milk appears to stimulate the secretion of pregastric esterase to a greater extent than does the ingestion of hay and grain. Wise *et al.* (14) reported that the secretion of pregastric esterase decreased progressively when milk was fed continuously, 25 lb. as five 5-lb. increments. Thus, the very young calf, consuming a smaller amount of milk than an older animal, would probably secrete more enzyme per unit of milk ingested. In addition, the secretion of gastric and postgastric lipases in other young mammals is believed to be low (1, 2, 3, 5, 8). If such is true also for the calf, the relative importance of pregastric esterase would be increased accordingly.

The present study emphasizes the need for further investigation of pregastric esterase, its nutritional significance, and factors affecting its secretion.

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EFFECTS OF CONTINUOUS NURSING, LENGTH OF THE NURSING PERIOD, AND RATE OF MILK CONSUMPTION ON THE SECRETION OF PREGASTRIC ESTERASE BY CALVES[1, 2]

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SUMMARY

The effects of continuous nursing, length of the nursing period, and rate of milk consumption on the secretion of pregastric esterase by calves were investigated. When 50 lb. of milk was fed continuously, as ten successive 5-lb. increments, the enzyme content of each increment decreased progressively. The quantity of enzyme secreted into a single 5-lb. unit of milk, however, was increased markedly by increasing the length of the nursing period. Increasing the rate of milk consumption appeared to stimulate the output of pregastric esterase slightly. The samples of sham-fed milk collected in the morning contained more enzyme than did those collected in the afternoon.

A knowledge of factors affecting the secretion of digestive enzymes is of importance in nutrition. Involvement of pregastric esterase, a lipolytic enzyme secreted by calves, in fat hydrolysis has been detected (4, 5, 6), but its physiological significance has not been established. Among factors that apparently are related to the secretion of this enzyme into milk are individuality of animals; system of feeding, drinking vs. nursing; quantity of milk consumed in continuous nursing (5, 6, 8); and length of the nursing period (7). Neither the age of the calf nor the diet upon which it is sustained, milk vs. hay-grain, however, seems to affect the quantity of pregastric esterase secreted into milk consumed by nursing. On the other hand, secretion effected by the consumption of either hay or grain per se appears to be very low compared to that stimulated by the nursing of milk (8). Thus, in view of the possible physiological importance of pregastric esterase in the alimentary lipolysis of dietary fat, further study of factors affecting its secretion was conducted.

EXPERIMENTAL PROCEDURE

General procedures. Nine calves, which were rumen-fistulated at 2 to 3 wk. of age, were used in this experiment. Six of these animals, three being sustained on a diet of hay and grain and the others on a diet of whole milk, have been described (8). The three additional calves used in the present study also were restricted to a whole-milk diet.

Secretion of pregastric esterase by the experimental subjects was determined by nipple-feeding nonfat milk (1 lb. of nonfat milk solids per 9 lb. of water), collecting the milk-saliva mixture from the cardial end of the esophagus,

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and measuring the enzyme activity thereof. Two different assay procedures were used. The first method, in which milk fat is employed as the substrate, was used as described previously (8), except for minor modifications. The second method, a photometric technique (3), utilizes 2-naphthyl laurate as the substrate. The pH, temperature, and length of incubation for the latter procedure were 4.4, 35° C., and 40 min., respectively. Although the pH optimum of pregastric esterase for milk fat is 6.1 (7), it shifts to approximately 4.7 under the conditions of the photometric procedure. In the presentation of results, hydrolysis of the milk fat is reported as μM of free fatty acid per 5 ml. of incubation mixture, and that of the 2-naphthyl laurate, as γ of 2-naphthyl. The extent to which sham-fed milk was diluted with saliva also was measured (8) during part of the present study.

Relationship between continuous nursing and secretion of pregastric esterase. The primary objective in the initial phase of this experiment was to study the relationship between the quantity of milk consumed and the secretion of pregastric esterase. Thus, the calves were sham-fed 50 lb. of nonfat milk as ten consecutive 5-lb. increments from a plastic nipple-feeder.[3] No attempt was made to control the rate of intake, although the time required to consume each increment was measured. Each collected increment was mixed thoroughly, and a portion of it was diluted fifty-fold with distilled water. Subsequently, 1 ml. of this diluted material was assayed by the photometric procedure for pregastric esterase. Twenty sets of such observations, involving all nine calves, were made over an age range of 37 to 243 days (37 to 143 for the animals fed only whole milk, and 118 to 243 for those sustained on hay and grain).

The results, summarized in Table 1, show that the level of pregastric esterase was always highest in the first unit of sham-fed milk and progressively lower in the nine succeeding units. The rate of decrease, however, was most pronounced during the time that the first three 5-lb. units were being consumed. This pattern of secretion was similar for all calves, regardless of either their age or diet. The difference in nursing time between calves fed hay and grain and those receiving whole milk seemed to be a reflection of individual nursing habits rather than a function of diet. For most of the calves, however, the nursing time per 5 lb. of milk increased after the first 25 lb. had been consumed. This reduction in rate of intake presumably was due to fatigue.

Relationship between length of the nursing period and secretion of pregastric esterase. Previous work (7), in which nursing time was measured but not controlled, indicated that the level of pregastric esterase in sham-fed milk tended to increase whenever the time required to consume the milk increased. This relationship was apparent, however, only when different observations on the same calf were considered. Among individual calves, the nursing habits, as well as the secretion of pregastric esterase, varied appreciably. Thus, calves that nursed most slowly did not necessarily secrete the greatest amount of this enzyme.

[3] Nurs-A-Calf feeder, supplied through the courtesy of R. K. Kuss and Company, Inc., Findlay, Ohio.

TABLE 1

Effect of sham-feeding 50 lb. of milk, as ten successive 5-lb. increments, on the sustained secretion of pregastric esterase

Increment number	Whole milk diet[a]			Hay-grain diet[b]		
	Hydrolysis of substrate	Per cent of first increment	Nursing time	Hydrolysis of substrate	Per cent of first increment	Nursing time
	(γ 2-naphthyl)		*(min.)*	*(γ 2-naphthyl)*		*(min.)*
1	95.0	100	2.63	83.1	100	3.35
2	45.5	48	2.47	34.5	42	3.35
3	33.5	35	2.55	22.9	28	3.38
4	29.2	31	2.62	18.5	22	3.60
5	25.8	27	2.57	16.0	19	3.50
6	22.8	24	2.68	14.8	18	4.07
7	20.5	22	2.77	13.3	16	4.25
8	19.3	20	2.97	12.1	15	4.28
9	18.0	19	3.03	10.8	13	4.27
10	16.6	18	3.12	9.0	11	4.42
		Total	27.4			38.5

[a] Mean of 14 observations involving six calves.
[b] Mean of six observations involving three calves.

In the present study, the relationship between ingestion time and secretion of pregastric esterase was investigated more fully by controlling the time required to consume a standard amount (5 lb.) of milk. Experimental subjects for this phase of the investigation were two calves that had been restricted to whole milk. All observations were made during the age range of 60 to 90 days. The different nursing times, averaging 0.7, 2.3, 5.3, and 10.8 min. in length, are designated hereafter, respectively, as very short, short, long, and very long. For the very short time, accelerated intake was achieved by enlarging the nipple orifice. For the other nursing times, the milk was permitted to flow at controlled rates into the nipple-feeder from a second container, thereby regulating the time required for consumption.

An evaluation of the treatments was accomplished in three separate periods, during which only two treatments were studied at any one time. Observations were made twice daily (8 A.M. and 4 P.M.) on consecutive days. The calves were rotated between treatments (Table 2), so as to minimize the confounding of treatment and other main effects and to average out possible carry-over effects from one collection to another. Immediately after the sham-fed samples were collected, each calf was given its regular allowance of whole milk.

TABLE 2

Schedule of rotating calves between treatments

	Day			
	1		2	
	Treatment	Calf	Treatment	Calf
Morning	A	1	A	2
	B	2	B	1
Afternoon	A	2	A	1
	B	1	B	2

Both of the assay procedures mentioned previously were used in analyzing the sham-fed samples collected during the first comparison period, but only the photometric technique was used subsequently. The incubation mixture for the milk-fat assay was as follows: 10 ml. of evaporated milk, 5 ml. of sham-fed nonfat milk, 5 ml. of unfed nonfat milk, and 3 ml. of buffer. Following incubation, 5 ml. of this mixture was removed for analysis. For the photometric procedure, 1 ml. of a fifty-fold dilution of the sham-fed milk was used per assay. The results of this phase of the experiment are presented in Table 3.

TABLE 3

Effect of nursing time on the secretion of pregastric esterase into 5 lb. of sham-fed milk

Comparison period		Nursing time designations	No. of observations	Nursing time	Consumption rate	Hydrolysis of substrate		Dilution of milk with saliva
						2-naphthyl	Fatty acid	
No.	Duration							
	(days)			*(min.)*	*(lb/min)*	*(γ)*	*(μM)*	*(%)*
I	6	Short	12	2.32	2.16	82.9	62.4	1.78
		Long	12	5.35	0.93	106.1	68.7	2.18
II	2	Very short	4	0.73	6.85	38.7	....	....
		Short	4	2.30	2.17	85.1	....	....
III	2	Long	4	5.28	0.95	89.6	....	....
		Very long	4	10.82	0.46	101.2	....	....

In the first comparison period, the samples from the long nursing time exhibited a higher level of pregastric esterase activity than did those from the short nursing time. This difference was statistically significant at the 1% level of probability for the photometric procedure, whereas it was significant only at the 5% level for the other assay method. Since the milk-fat assay technique appeared to be the less sensitive of the two procedures, it was discontinued during the remainder of this phase of the experiment. In each of the two subsequent comparison periods, the longer of the two nursing times always had a higher level of pregastric esterase.

To evaluate this relationship further, the mean enzyme activity of the very long samples was assigned a value of 100, and that of the other samples was adjusted proportionately. These relative values (Figure 1) indicate that the total secretion of pregastric esterase increased markedly as nursing time was extended. When the duration of the nursing period was slightly less than 1 min., the total output of enzyme was only one-third of that observed when the time was nearly 11 min. Although the rate of esterase secretion appeared to diminish with time, previous observations (Table 1) indicate that calves are still able to secrete small quantities of pregastric esterase even after nursing continuously for nearly 40 min. The volume of saliva secreted into the sham-fed samples, which was quite variable, did not seem to be related primarily to the length of the nursing period.

Relationship between rate of milk consumption and secretion of pregastric esterase. Although length of nursing time was the primary factor under investigation in the preceding phase of this study, one of the accompanying variables was a decrease in the rate of milk ingestion as the nursing time increased.

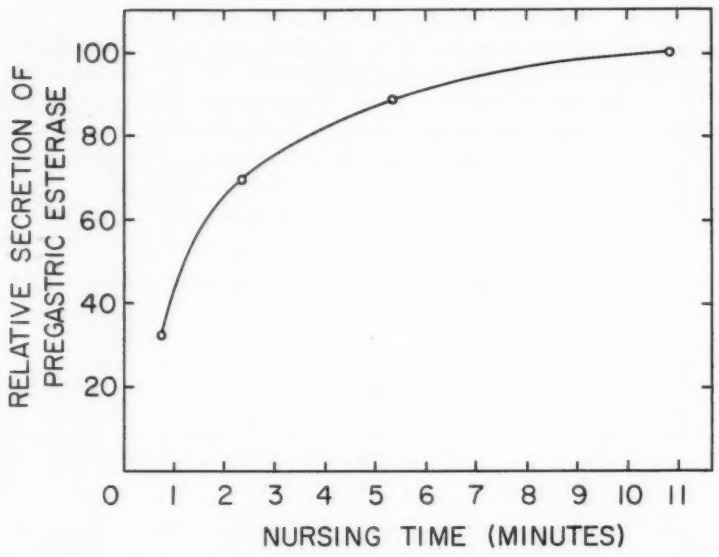

FIG. 1. Effect of nursing time on the relative secretion of pregastric esterase into 5 lb. of sham-fed milk.

Thus, the effect of consumption rate per se on the secretion of pregastric esterase was investigated in the third phase of this experiment. Two consumption rates, controlled as described previously, were studied and correspond to those associated with the previous short and long nursing times. Length of nursing was held constant, 4.0 min. The quantity of milk consumed was determined by weighing the unconsumed portion and subtracting it from the total.

Both assay procedures, the milk-fat and the photometric, were employed. To obtain an expression of the total quantity of pregastric esterase secreted during the nursing period, the assays were conducted as follows: In the milk-fat procedure, the incubation mixture consisted of 10 ml. of evaporated milk, 3 ml. of buffer, 1 ml. of sham-fed milk per pound of milk consumed, and sufficient unfed nonfat milk to bring the total volume to 23 ml. In the photometric method, 0.1 ml. of sham-fed milk per pound of milk consumed was added to 49 ml. of water. The total volume was brought to 50 ml. with unfed nonfat milk, and 1 ml. of this mixture was used per assay. Thus, in each method, a definite percentage of the total quantity of enzyme in the sham-fed sample was taken for analysis.

Observations were made twice daily for six days on the two calves used previously, which were rotated between treatments as indicated previously. The results, summarized in Table 4, show that when nursing time was held constant, the total secretion of pregastric esterase was greater when milk was consumed at the fast rate than when it was consumed at the slow rate. The differences in response between rates of intake were statistically significant at

the 5% level of probability for both methods of assay. The means by which consumption rate affects the total output of pregastric esterase is obscure. This variable per se, however, probably is not so influential as nursing time per se. In the second phase of this investigation (Table 3), a combination of the long nursing time and slow consumption rate effected greater secretion than did a combination of the short nursing time and fast consumption rate.

TABLE 4
Effect of consumption rate on the secretion of pregastric esterase into sham-fed milk

Duration of comparison period	Consumption rate designations	No. of observations	Nursing time	Milk consumption		Hydrolysis of substrate	
				Total	Rate	2-naphthyl	Fatty acid
(days)			*(min.)*	*(lb.)*	*(lb/min)*	*(γ)*	*(μM)*
6	Fast	12	4.00	9.1	2.28	57.0	92.5
	Slow	12	4.00	4.0	1.00	45.7	88.8

Relationship between daily feeding schedule and secretion of pregastric esterase. All of the data were examined further in an effort to detect other factors that may affect the secretion of pregastric esterase. Insofar as could be determined, there appeared to be either little or no carry-over effect of treatments from one collection to the next. As indicated in the experimental procedure, each calf was fed its regular allowance of whole milk immediately after collecting the sham-fed samples. Thus, the combination of these two successive nursing periods probably depleted each calf of its pregastric esterase to approximately the same extent, regardless of treatment. It was noticed, however, that the samples collected in the morning usually contained more pregastric esterase than did those collected in the afternoon (Table 5). When all of the photometric data were pooled and subjected to an analysis of variance, the morning-afternoon difference was found to be statistically significant at the 1% level of probability. For the milk-fat assays, however, no marked difference was evident, which perhaps was due to the lower sensitivity of this method. The higher levels of enzyme for the morning samples may be related to the longer interval of time between afternoon and morning feeding, 16 hr., as compared to 8 hr. between morning and afternoon feeding. The period of

TABLE 5
Mean pregastric esterase activity of sham-fed samples of milk collected in the morning compared to those collected in the afternoon

Time of collection	Photometric assays				Milk fat assays	
	Nursing-time comparisons			Consumption rate comparison	Nursing-time comparison	Consumption rate comparison
	Short vs. long	Very short vs. short	Very long vs. long	Fast vs. slow	Short vs. long	Fast vs. slow
	(γ 2-naphthyl)				*(μM fatty acid)*	
Morning	98.2	66.2	99.5	56.6	67.4	89.6
Afternoon	90.8	57.6	91.3	43.1	63.7	91.7

8 hr. may not be adequate for the calf to replenish completely its supply of pregastric esterase. The possibility that other factors may be involved is not excluded, however.

DISCUSSION

The observed decrease in the secretion rate of pregastric esterase as milk is fed continuously substantiates previous observations (6) and suggests that this enzyme is accumulated during the interval between feedings. The continuing presence of esterase in sham-fed milk, however, even when a large volume is consumed, suggests that this enzyme is also synthesized during the nursing period.

Inasmuch as pregastric esterase is secreted continuously during milk ingestion, the finding that a longer nursing period per unit of milk increased the quantity of enzyme secreted into that unit was in accord with expectations. Consideration of the practical aspects of this finding raises the question of whether or not the calf that consumes milk slowly digests the fat contained therein any more effectively than the calf that consumes milk very rapidly. If such is the case, this difference apparently does not manifest itself in the growth of the calf. Evidence in recent years indicates that the feeding of milk from an open pail promotes growth equal to that obtained when the milk is nipple-fed (1, 2), even though the latter system requires a longer time for milk consumption. The drinking of milk, however, does stimulate the secretion of pregastric esterase, although in smaller quantities than that induced by nursing (5, 6).

The means by which a more rapid consumption rate per se increased the output of pregastric esterase is not clear, although it may be primarily a physical phenomenon, i.e., one of washing the oral and pharyngeal secretions more completely from their respective areas. In considering the total secretion of pregastric esterase per unit volume of milk, however, the increase effected by rapid consumption is more than offset by the decrease effected by the short nursing period. Thus, the length of nursing is of primary importance.

Although the relative importance of pregastric esterase to the calf can not be assessed from the present study, the need for further research in this area is apparent, particularly in view of the fact that the pregastric secretions generally have not been considered to be of importance in fat digestion. Special consideration should be given to the role that this enzyme plays in the alimentary hydrolysis of dietary fat and to the various factors affecting the extent of this action.

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RELATIVE GROWTH AND APPEARANCE OF YOUNG DAIRY CALVES FED TWO LEVELS OF MILK WITH A SIMPLE OR COMPLEX CALF STARTER

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SUMMARY

One hundred and sixty Holstein heifer calves were used on a 7-wk. factorial growth experiment to compare 350 vs. 250 lb. of whole milk and a simple vs. a complex dry calf starter. The experiments were conducted during a fall and summer season with calves housed in four calf units, consisting of pens and tie stalls. Calves consuming 350 lb. of whole milk gained 1.20 lb. per day, which was significantly ($P < 0.01$) greater that the 1.12 lb. per day for the 250-lb. group. Starter consumption was significantly greater for the 250-lb. group. There was no difference between starters in either consumption or gain. The TDN content of both starters was 69%. Rate of gain was less for the fall season. The tie stalls were less desirable in terms of gain during the colder months. Ninety-six per cent of the calves which gained over 1 lb. per day were thrifty and smoothly fleshed. This gain is proposed as a practical minimum for Holstein heifers to 7 wk. of age. Eighty per cent of the calves on the 350-lb. milk level and 72% of those on the 250-lb. level achieved this rate of gain. The 250-lb. milk simple starter system was the least expensive, due primarily to the lower cost of the milk required.

In preparing general recommendations for the rearing of young dairy calves, it was impossible from existing published data to conclude that any one feeding system was the best. This was not to say, however, that all of the systems evaluated were identical, but merely that each had certain advantages and disadvantages which varied in importance, depending upon the dairyman's individual requirements. It appeared that it would be useful in the preparation of such recommendations to be able to describe the performance parameters of several successful systems in absolute as well as relative terms of growth, feed consumption, and calf appearance. The dairyman could then be advised to select one that was best fitted to his requirements in terms of performance and cost, as well as to his skill and available resources, in terms of feeding and management.

An examination of the literature, our own as well as that of others, revealed that there were inadequate data to make such a complete recommendation. In many cases, the experiments reported contained too few animals to detect important treatment differences and many were not repeated at different seasons or under different management conditions. In addition, it was noted that the level of performance was often below that considered to be adequate or normal. The feeding regime which results in poor performance is obviously of little value, even though it may well be significantly superior to another poor treatment.

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As a step towards obtaining adequate information to describe various successful feeding systems, the following study was conducted. The standard Cornell system of 350 lb. of whole milk (20) was compared with 250 lb. These levels were in turn fed with either a simple- or complex-formula calf starter. While simple type starters have been recommended with liberal levels of milk feeding (13, 16), they have not been extensively tested at milk levels below 350 lb. The general use of complex-formula calf starters stems from the early work of Savage and Crawford (17), who reported excellent gains when a fortified starter was fed to Holsteins along with 350 lb. of whole milk. In this report they did not, however, make any direct comparisons with a simple formula. More recent data have shown that with similar levels of milk there has been no significant advantage for a complex over a simple formula (2, 4, 7, 9, 11). Examination of those reports published prior to initiating this study suggested that there was need for additional information, when smaller amounts of milk were fed.

Eaton *et al.* (1) recently reported that a well-designed experiment should have 20 to 25 calves per group, to detect a 20% difference in rate of gain to 7 wk. This figure coincides with the estimate based on our own data. In addition, our figures suggest groups of at least 40 calves, in order to detect a similar difference in dry calf starter consumption. The following study was, therefore, designed with at least these numbers of animals per group.

EXPERIMENTAL PROCEDURE

A total of 160 heifer calves was purchased from four local auction markets. They weighed from 85 to 95 lb., showed Holstein-Friesian characteristics, still possessed a short, dried length of umbilical cord, and had horn buttons which could barely be felt. They were assumed to be three days old at purchase.

Eighty calves were allotted at random to treatments arranged as a $2 \times 2 \times 4$ factorial with 350 and 250 lb. of milk, simple and complex calf starter,[1] and four housing units. The calves were housed in several units, in order to give a broader management base upon which to evaluate the diets. Two of these units were located in the Cornell Blair Barn and consisted of 20, 4- by 6-ft. pens (Pens B) alternated with 20, 3 by 4-ft. tie stalls (Ties B). The other units were of 20 pens (4 by 7 ft. or 7 by 6 ft.) located 2 and 5 mi. from the campus, respectively, in the Mitchell (Pens M) and Warren Farm (Pens W) calf barns. The first experiment of 80 calves was conducted from June 25 through August 3, and the second from October 10 through November 18, 1957.

[1] Composition (pound per ton), *Complex*: flaked corn, 352.5; wheat bran, 200; crushed oats, 350; *linseed meal, 200; *soybean oil meal (44% protein), 360; *alfalfa meal, 140; cane molasses, 160; *dried skimmilk, 100; *corn distiller's solubles, 100; *irradiated yeast, 0.5 (9,000 I.U. vitamin D/g); *vitamin A palmitate, 4 (4,500 I.U./g); *dicalcium phosphate, 10; *ground limestone, 10; *iodized salt, 10; Aurofac 10, 3; 2 g. $CoSO_4$ added to each ton. *Simple*: from Holter (7), cracked corn, 580; wheat bran, 300; crushed oats, 500; soybean oil meal, 600; dicalcium phosphate, 10; iodized salt, 10.

* These ingredients pelleted with half the molasses; remaining ingredients and pellets mixed with remaining molasses.

Dry feed (hay and starter) was offered to the calves after the second day of the experiment. During the first week a handful of starter was placed in each calf's mouth immediately after it finished its milk. As the experiment progressed, consumption was limited to 4.0 lb. per day and was weighed back if more than 2 lb. accumulated in the feed box. This weigh-back was replaced with fresh feed. The hay was second-cutting alfalfa, grass mixed-hay and was chopped into approximately 4-in. lengths. Fresh water in a pail was continuously available and changed daily. The proximate composition of the hay and starters appears in Table 1.

TABLE 1
Chemical composition of the calf starters and hays fed[a]

	Dry matter	Crude protein	Ether extract	Ash	Crude fiber	Nitrogen-free extract
Hay	88.3	16.8	1.5	6.3	27.0	36.7
Simple starter	89.8	18.8	2.4	7.0	8.7	52.9
Complex starter	88.0	20.7	2.0	4.6	8.2	52.5

[a] Milk was Cornell herd milk testing approximately 3.6% fat from Holstein cows.

Disease control consisted of giving 12 g. of sulfathaladine orally to all calves upon arrival, and feeding 30 mg. of chlortetracycline daily in the milk. Pens were cleaned daily and fresh wood shavings added. If scours occurred, the daily milk allowance was reduced. Recovery was followed by a gradual increase in feed. Milk withheld was fed to the calf as soon as she could safely consume it.

TABLE 2
Milk feeding schedules used in the experiment (pounds per feeding)

	350 lb.	250 lb.
Birth to 3 days	Dam	Dam
Fourth to seventh day	4.0	4.0
Second week	4.5	4.5
Third week	5.0	5.0
Fourth week	4.5	3.5
Fifth week	3.5	2.5
Sixth week	3.0	....
Seventh week	2.0	

Milk was fed in open pails at the rates shown in Table 2. It should be noted that the 350-lb. schedule terminates at 7 wk., whereas the 250-lb. calves were weaned at 5 wk. During the first 3 wk., both schemes provided the same amount of milk, approximately equal to the lower level of the Morrison (13) recommendation for TDN. Previous efforts to foster substantial hay and starter consumption during the first 3 wk. by feeding the calves only one-half of their nutrient requirements as milk were unsuccessful, since little dry feed was eaten and the calves lost weight. As a result, the present uniform schedule was adopted, based on the observation that significant dry feed consumption would occur only after 2 to 3 wk. of age.

Weights were taken with a walk-on platform scale, and wither height was measured using a swinging horizontal bar which also slid vertically. This bar was permanently mounted on the weighing crate. Three-day weights were averaged at the beginning of the experiment and at 7 wk. Wither height was measured for three days at the beginning and one day at the close of the study. At 7 wk. general fleshing was evaluated by one of us (H.N.H.) without noting the identity and weight gains of each calf. Of many possible observations, only fleshing and thriftiness were noted, because it appeared from Hemken's (3) study of calf growth where a score was given to relative fleshing, general appearance, and two other criteria that the former two varied together to approximately the same magnitude.

Analysis of variance and Hartley's sequential test (19) were used to locate differences among treatment means.

Standard digestion trials were carried out at 16 wk. of age, using a total of six calves, two receiving each of the starters and two receiving hay alone. Where used, the starters constituted 60% of the ration. A 2-wk. constant intake and ten-day collection period were used. Starter TDN was calculated by difference.

RESULTS AND DISCUSSION

Tables 3 and 4 summarize the results of the experiment, and Table 5 contains the mean square values for treatment and error effects. The following discussion of treatment differences includes only those which were significant at the 5% level or less.

The 350-lb. milk level caused 8% greater weight gains and 11% greater gain in height at withers than 250 lb. The former group also consumed 11% more total calculated TDN (100 vs. 90 lb.), even though they ate 16% less grain and hay. In effect, the 250-lb. group failed to compensate completely by increased hay and starter consumption for the lower level of milk they received. This trend is consistent with previous results at this station (21). The rate of gain of the 350-lb. group is consistent with some data reported from Cornell (3, 7, 18), but is greater than other studies in which the housing was at best mediocre (6).

The simple starter was consumed equally as well as the complex. The growth by the calves showed a small but statistically significant advantage for the simple starter. This difference in growth appears to be too small to be practically significant, however. The similarity of the two starters agrees with the experiments cited previously, as well as more recent reports in which Holsteins were fed 39 lb. of milk replacer (10) or Jerseys received about 120 lb. of milk (14).

TDN values of both starters were the same, 69, when calculated by difference. The hay alone was 42, a value substantially lower than previously noted for 16-wk.-old calves fed a similar hay (5). The TDN of the mixture of 60% starter and 40% hay was 58%.

The interaction of milk times starter shown in Table 4 was statistically significant, but was numerically too small to carry much practical significance.

TABLE 3

Growth and feed consumption of Holstein calves fed the various diets

Criterion	Milk level *(lb.)*		Starter		Replicate		Location			
	350	250	Simple	Complex	Summer	Fall	Tie-stalls B	Pens B	Pens M	Pens W
No. of calves	80	80	80	80	80	80	40	40	40	40
Av. daily gain *(lb.)*	1.21**	1.12	1.18*	1.15	1.23**	1.10	1.14[a]	1.23[a]	1.10[a]	1.18[a]
Total height gain *(in.)*	3.1**	2.8	3.0	3.0	3.1	2.9	2.9	3.0	2.9	3.1
Total starter consumed *(lb.)*	49	59**	56	52	61**	47	54	62	43[b]	57
Total hay and starter consumed *(lb.)*	61	73**	68	66	70**	63	63[b]	72	63[b]	70
Percentage of dry feed consumed as starter	80	80	83	77	85**	74	83	86	67[b]	82
TDN consumed	100*	90								

[a] The four means were compared, using Hartley's sequential test of significance (19) ($P < 0.05$). Significantly different treatments are above different lines.

Pens B	Pens W	Tie-stalls B	Pens M

[b] Values indicated are significantly different ($P < 0.05$) than others in group using the same test as [a] above.

* $P < 0.05$; ** $P < 0.01$.

TABLE 4

Summary of the statistically significant interactions observed with dairy calves (mean square values in Table 5)

Interaction	Observation	No. of calves	Treatments							
Milk × starter			350 lb.				250 lb.			
			Simple		Complex		Simple		Complex	
	Av. daily gain *(lb.)*	40	1.25		1.16		1.09		1.14	
	Height at withers *(in.)*	40	3.3		3.0		2.8		2.9	
Season × starter			Summer				Fall			
			Simple		Complex		Simple		Complex	
	Av. daily gain *(lb.)*	40	1.20		1.25		1.15		1.05	
	Height at withers *(in.)*	40	3.0		3.2		3.1		2.7	
	Starter consumed *(lb.)*	40	58		63		53		41	
	Hay + starter consumed *(lb.)*	40	66		74		67		56	
Season × milk			Summer				Fall			
			350 lb.		250 lb.		350 lb.		250 lb.	
	Av. daily gain *(lb.)*	40	1.26		1.19		1.16		1.04	
Season × farms			Summer				Fall			
			Pens B	Pens M	Pens W	Tie B	Pens B	Pens M	Pens W	Tie B
	Av. daily gain *(lb.)*	20	1.26	1.22	1.20	1.23	1.20	0.97	1.17	1.07
	Starter consumed *(lb.)*	20	65	55	62	61	60	30	53	46
	Percentage of dry feed consumed as starter	20	88	81	86	85	84	78	53	82

TABLE 5

Mean square values[a] and coefficients of variation derived from a study of 160 Holstein calves fed several diets

Source of variation	d.f.	Daily gain (lb.)	Total height gain (in.)	Total starter consumed (lb.)	Total hay and starter consumed (lb.)	Per cent of dry feed consumed as starter
Farms (F)	3	0.200**	0.297	15,214.0**	1,158.0*	3,276.1**
Milk (M)	1	0.257**	3.561**	4,478.4**	6,043.5**	272.7
Starter (S)	1	0.110*	0.155	387.5	27.8	1,072.9
Replicate (Season, R)	1	0.529**	0.748	7,066.8**	2,314.0**	6,171.6**
F × M	3	0.012	0.103	290.2	2,135.0**	234.9
F × S	3	0.035	0.068	498.1	10.8	127.7
F × R	3	0.141**	0.350	817.0*	183.5	1,528.6**
M × S	1	0.106*	2.470*	556.5	26.1	211.6
M × R	1	0.082*	0	164.3	27.7	14.2
S × R	1	0.381**	3.270**	2,407.9**	2,105.0**	227.9
Within	128	0.0199	0.479	293.2	298.3	296.7
Coefficient of variation (%)		12	23	32	26	22

[a] Second- and third-order interactions were all insignificant and are omitted.
* $P < 0.05$; ** $P < 0.01$.

The growth and feed consumption of the calves during the fall replicate were significantly less than in the summer study (Table 3). This is not completely explainable, as there was a low incidence of respiratory and digestive upsets during both seasons, and the lower mean barn temperature in the fall (55 vs. 76° F.) would be expected to stimulate feed consumption. A partial explanation appears to be the lowered complex starter consumption which occurred in the fall. This reduction in intake was four times the reduction in simple starter consumption (22 vs. 5 lb.). A different lot of starter was used for each replicate and some change may have occurred in the complex starter while the simple starter remained more uniform. A less vigorous group of calves was apparently studied in the fall than in the summer. This is shown by the reduced consumption of both starters which took place each week in the fall replicate compared to the summer. These average reductions in pounds of starter per week were as follows, beginning with the 2nd wk.: 0, 0.4, 0.9, 1.7, 3.0, 4.0, 3.0. Their lack of vigor may have been due to the fact that the month in which they were born follows the relatively poor pasture months of July to September in New York State. The season times milk interaction was significant ($P < .05$) but not interpretable. The season times barns interaction was also significant. The calves in the fall replicate grew poorly in the case of Pens M and Tie Stalls B. Inexperienced and disinterested calf feeders were apparently the cause of the reduction during the fall in Pens M; however, the reduction seen in the tie stalls took place under the care of the same personnel which produced excellent results the previous summer. From these observations, it appears that there was something about the tie stalls which caused them to be less desirable than the pens during the colder weather.

A summary of the relationship of the appearance of the calves to their weight gains constitutes Table 6. The number which were smoothly fleshed and

TABLE 6

Effect of two levels of milk on the rate of gain and general appearance of calves to 7 wk. of age

Rate of gain *(lb. per day)*	Milk level			
	250 lb.		350 lb.	
	No. of calves	No. of thrifty calves	No. of calves	No. of thrifty calves
0.65 to 0.84	13	5	3	0
0.85 to 0.90	3	3	3	2
0.91 to 0.99	6	5	9	7
1.00 to 1.20	32	29	20	20
Over 1.21	26	26	45	42
Total	80	68	80	71

thrifty in appearance is noted. The minimum standard for a smoothly fleshed and thrifty animal required that the vertebrae give no rough appearance; the ribs be evenly covered with a medium layer of flesh and show only slight depression between them; that hip bones and rump be medium-fleshed and smooth, with no abrupt hip protrusion or hollowness in the region of the thurls. Such an observation should have a close relationship with the nutrition of the animal and was an attempt to classify what a dairyman might commonly use as a minimum standard for assessing the nutritional adequacy of his calf-feeding program. The proportion of the calves which were considered well-fleshed and thrifty was 96% when gains were over 1 lb. per day. From 0.85 to 1.0 lb. per day, 86% were well-fleshed. Below 0.85 lb. per day, the per cent of well-fleshed calves dropped precipitously to 31%. The small numbers of animals in the lower brackets make percentage figures for these tenuous at best. From these observations it would appear that a good guarantee of visual satisfaction should be possible for Holstein calves gaining about 1 lb. per day or more. This relationship of gain per day and appearance should be examined on a broader scale by others, to see if the opinion of the authors is widely applicable. It is worthy of note that the level of 1 lb. per day is slightly higher than 0.9, which is the Ragsdale (15) and Beltsville (12) standard for this age. This is understandable when one considers that these standards are averages of the growth of exceptionally good, as well as very poor calves. The rate of 1 lb. per day is considerably below the maximum gain of 2.0 lb. per day which is possible for Holstein heifers fed milk ad libitum (8, 13).

Seventy-two per cent of the calves fed 250 lb. of milk gained 1 lb. per day or more, and 80% of the calves fed 350 did likewise. Of interest also is the fact that 45% of calves receiving 350 lb. of milk gained over 1.20, while 25% of those receiving 250 lb. gained this rapidly. Although both systems gave excellent results as an average, approximately 27% of the calves on the 250-lb. level and 20% of those on the 350-lb. level gained less than 1 lb. per day. It appears, therefore, that these poorer calves would have profited by additional milk if the appearance standard is to be attained by all calves. The decision as to whether to do this lies with the dairyman.

The 250-lb. scheme using the simple calf starter was substantially less expensive than the 350-lb. complex starter system. The difference in cost was

primarily due to the reduction in milk fed, with only a small proportion of the total attributable to differences in the price of the two starters.

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EFFECTS OF FEEDING AUROFAC[1] UPON THE GROWTH OF SINDHI × JERSEY MALE CALVES TO SIX MONTHS OF AGE

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SUMMARY

Calves were fed 5 g. Aurofac per day, first in whole milk, then in skimmilk, from four days to 4 wk. of age. From 4 to 24 wk. Aurofac was fed in grain at the rate of 20 lb. per ton of feed. The calves fed Aurofac gained 18% more in weight and 23.6% more in height at withers than untreated calves during a 24-wk. feeding period. These differences are statistically significant at the 1% level of probability.

The influence of Aureomycin on the growth and well-being of young dairy calves in the United States has been well established and reviewed by Lassiter (4). However, only a limited amount of information is available regarding its use in India. Bahri *et al.* (1) fed Aurofac to Sindhi, Gir, and Crossbred calves at the rate of 10 g. per 2.5 lb. dry feed, and reported an average daily gain of 0.82 lb. per day for calves receiving Aurofac and 0.48 lb. per day for the controls. Reed and Velu (8) fed 20 mg. Aureomycin daily to calves from four days to 12 wk. of age and reported increased growth rate, but the difference was not statistically significant. These calves were fed whole milk to 25 days and skimmilk from 25 days to 12 wk. of age. Lassiter *et al.* (5) reported a highly significant increase in gains of Holstein calves when fed aureomycin to 12 wk. of age.

Since very little work has been done in the feeding of antibiotics under Indian conditions, and since a preliminary study showed some favorable results from the feeding of Aureomycin, it was considered desirable to further investigate the effect of the use of this antibiotic upon the growth of calves raised on skimmilk from 15 days of age. Results of such an investigation are reported in this paper.

EXPERIMENTAL PROCEDURE

As male calves of approximately half Jersey and half Red Sindhi blood lines were dropped in the Allahabad Agricultural Institute herd after January 1, they were alternately assigned to one of two groups until six comparable pairs were present. All calves were left with their dams for 24 hr. to receive colostrum. On the second day they were moved to one of two similar pens and colostrum was fed at the rate of 1 lb. per 10 lb. of body weight, through the third day. On the fourth day and thereafter until the 15th day all calves were

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[1] Aurofac–Substance containing 1.8 g. Aureomycin and 1.8 mg. of vitamin B_{12} per pound.

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TABLE 1

Average birth weight, cumulative gains at 2-wk. intervals, and final weight of calves at 24 wk. of age when fed Aurofac from four days of age

No. calves	Birth wt. (lb.)	Gains to indicated ages *(lb.)* 2 wk.	4 wk.	6 wk.	8 wk.	10 wk.	12 wk.	14 wk.	16 wk.	18 wk.	20 wk.	22 wk.	24 wk.	Final wt. *(lb.)*
						Untreated calves								
6	46 [a]±7.3	8 ±2.2	13 ±4.3	22 ±10.0	30 ±11.9	42 ±12.8	56 ±17.2	73 ±20.4	90 ±19.8	108 ±22.8	128 ±28.9	143 ±35.8	154 ±34.9	199 ±35.0
						Treated calves								
6	43 [a]±7.8	7 ±2.0	14 ±5.7	26 ± 6.9	38 ± 9.8	53 ±14.2	68 ±17.9	92 ±21.8	108 ±23.3	130 ±26.7	148 ±31.1	167 ±34.9	181 ±30.3	224 ±35.2

Analysis of variance of fortnightly rate of weight gains

Source of variance	Degrees of freedom	Sum of squares	Mean squares	Variance ratio	Significance
Groups	1	6,840.7	6,840.7	15.03	1%
Fortnights	11	416,822.5	37,892.9	83.24	1%
Groups × fortnights	11	2,924.8	265.9	0.58	N.S.
Error	120	54,627.9	455.2		

[a] Standard deviation.

fed whole milk from the Institute herd (approximately 4.5% fat) at the rate of 1 lb. for each 10 lb. body weight, with a maximum of 10 lb. per day until 12 wk. of age. The milk was fed in two equal portions daily, with two teaspoonfuls of shark liver oil added to the milk at each feeding.

Beginning on the fourth day and daily thereafter until calves were 4 wk. of age, one group of calves received 5 g. Aurofac[3] daily, added to the morning feeding of milk. From 4 wk. to 6 mo. of age this group of calves was fed Aurofac added to the grain mixture at the rate of 20 lb. per ton of feed. The grain mixture for both groups consisted of ground yellow maize 40 parts, gram (*Phaseolus aureus*) 15 parts, wheat bran 15 parts, ground nut cake 27 parts, and fish meal three parts, plus 3% regular Institute mineral mixture (salt 50%, steamed bonemeal 50%, plus 0.03 lb. KI per 100 lb.). This same grain mixture was offered free choice to both groups of calves until the close of the experiment. All calves had access to lucerne hay after the first day and to clean, fresh water throughout the experiment. Weight and height at withers were recorded at birth and each fortnight thereafter for each calf. Feed consumption was not recorded.

RESULTS AND DISCUSSION

The cumulative gains at fortnightly intervals and final weight of calves at 24 wk. of age are given in Table 1. After the 4th wk. the calves receiving

[3] Containing 1.8 g. Aureomycin and 1.8 mg. vitamin B_{12} per pound.

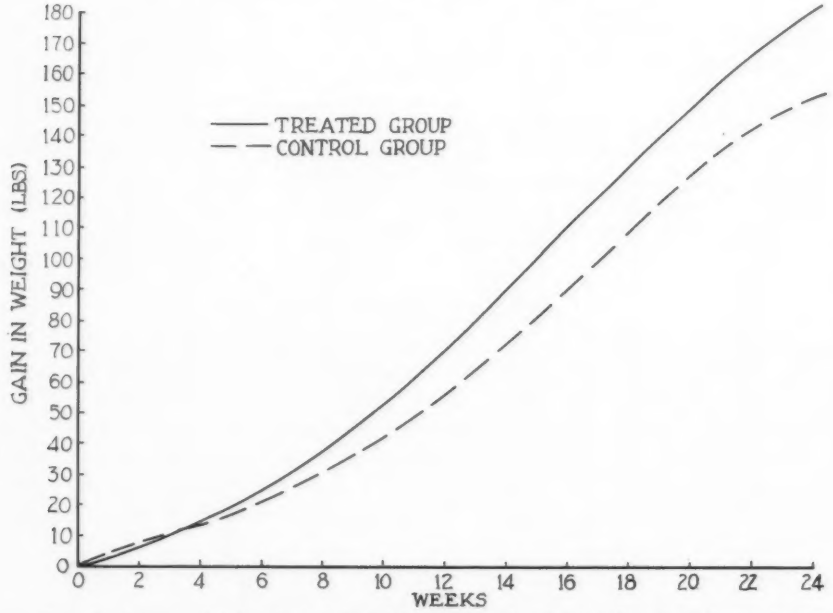

FIG. 1. Average total gains at fortnightly intervals of treated and control calves to 6 mo. of age.

TABLE 2

Average wither height at birth, cumulative gain in height at 2-wk. intervals, and final height of calves at 24 wk. of age when fed Aurofac from four days of age

No. calves	Wither height *(in.)*	2 wk.	4 wk.	6 wk.	8 wk.	10 wk.	12 wk.	14 wk.	16 wk.	18 wk.	20 wk.	22 wk.	24 wk.	Final height *(in.)*
						Untreated calves								
6	25.7	0.6	1.0	1.5	2.0	2.5	3.3	4.2	5.2	5.8	6.6	6.9	7.4	33.2
	[a]±1.1	± .7	±1.1	±0.4	±0.6	±0.6	±1.1	±1.2	±1.0	±1.1	±1.4	±1.5	±1.3	±1.6
						Treated calves								
6	24.6	0.7	1.3	1.8	2.5	3.5	4.3	5.3	6.5	7.1	8.3	8.9	9.2	33.8
	[a]±1.1	±0.8	±0.8	±0.9	±0.7	±1.3	±1.5	±1.7	±1.6	±1.1	±0.7	±0.5	±1.6	±1.5

Analysis of variance of fortnightly rate of height gains

Source of variance	Degrees of freedom	Sum of squares	Mean squares	Variance ratio	Significance
Groups	1	37.9	37.93	21.07	1%
Fortnights	11	997.3	90.7	50.38	1%
Groups × fortnights	11	12.9	1.2	0.66	N.S.
Error	120	220.7	1.8		

[a] Standard deviation.

Aurofac made greater gains than the control and treated calves. The average gain per calf for the test period for the control and treated calves was 153.5 lb. and 181.3 lb., respectively. This difference is statistically significant at the 1% level of probability. Figure 1 shows that after the 4th wk. the difference in gain between the two groups increased gradually until the 24th wk. These results are not in agreement with Loosli *et al.* (6) and Murdock *et al.* (7). Loosli *et al.* (6) reported a 22% faster growth the first 8 wk., but that at 16 wk. of age there was no further growth improvement from feeding Aureomycin. Murdock *et al.* (7) reported a growth response from Aureomycin feeding to 6 wk. of age, but by the time the calves were 12 wk. of age both the controls and Aureomycin-fed calves made similar gains. Bartley *et al.* (2) fed aureomycin supplement to calves to 22 wk. of age and obtained an increased growth response. Jacobson *et al.* (3) fed Aurofac to calves to 200 days of age and obtained increased growth, with the difference in weight between the treated and untreated calves being greater at 200 days than at 116 days.

The increase in height at withers at fortnightly intervals and final height at 24 wk. of age are given in Table 2. The calves receiving Aurofac increased 9.16 in. in height at withers and the untreated calves increased 7.41 in. in wither height during the 24-wk. period. This difference is statistically significant at the 1% level of probability.

Scours was not a problem in either group; thus, it was not possible to evaluate the effect of Aurofac in reducing scours.

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SOME FACTORS AFFECTING AGE AT PUBERTY AND THE FIRST 90 DAYS OF LACTATION IN HOLSTEIN HEIFERS[1]

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SUMMARY

Data from 184 Holstein heifers representing six sire lines and four systems of mating were analyzed to determine the genetic effects of sire-line and system of mating, and the environmental effect of season of birth on age at puberty. The mean age at puberty for this group of heifers was 345 days. Sire line and system of mating were found to affect significantly the age at which a heifer attained puberty. Age at puberty was correlated significantly with 6-mo. weight (-0.56, $P < .01$) and 6- to 12-mo. weight gain (-0.22, $P < .05$). After adjusting puberal ages for 6-mo. weight the significance of the effects of sire line and system of mating was removed. Calfhood scouring caused a delay in attainment of puberty which was significant in the fall-born heifers, in which the delay was 136 days. In the nonscouring O-O heifers (outbred animals from outbred dams), those born in the spring had the earliest age at puberty.

Age at puberty was correlated significantly with weight at first calving (-0.31, $P < .01$) and also with the 90-day milk production (-0.18, $P < .05$) and the average 90-day butterfat percentage (-0.31, $P < .01$) of the first lactation. Significant correlations existed for 6-mo. weight with calving weight (0.50, $P < .01$) and 90-day milk production (0.35, $P < .01$). Calving weight was correlated significantly with both the 90-day milk production (0.45, $P < .01$) and the average 90-day butterfat percentage (0.28, $P < .01$). Standard partial regression coefficients indicated that 90-day milk production was affected significantly only by calving weight (0.38, $P < .01$), whereas the average 90-day butterfat percentage was affected significantly by age at puberty (-0.25, $P < .05$) and calving weight (0.21, $P < .05$).

Significant amounts of variation in the age at which an animal attains puberty have been ascribed both to genetic and to certain identifiable environmental factors. Foote *et al.* (6) reported that in swine puberal age was nonadditively inherited and Warnick *et al.* (18) found differences in age at puberty among inbred lines of swine. These workers, as well as Robertson *et al.* (14), observed a negative association between age at puberty and 154-day weight. Donald and Anderson (3) observed in Holstein heifers that slower-growing animals reached puberty later.

Reid *et al.* (12) and Sorensen *et al.* (16) reported that heifers on a low or a high plane of nutrition had later or earlier puberal ages, respectively, than

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[2] Agent of the Dairy Cattle Research Branch, USDA.

heifers on a normal level of nutrition. In other studies on dairy heifers (2, 5, 8, 10) a lowered plane of nutrition delayed puberty as compared to a normal plane.

Season of birth has been shown in several species (7, 13, 14, 17, 19) to affect the sexual development, with the spring-born animals generally being the latest to reach sexual maturity.

The objectives of this study were to determine the effects of sire line, system of mating, season of birth, calfhood scouring, and growth rate on age at puberty in Holstein heifers. The possible effects of age at puberty on the first 90-day production in the first lactation of these heifers were also examined.

MATERIALS AND METHODS

The present study is in part complementary to the preliminary study of Hawk *et al.* (9). Data from 40 of the 67 heifers used in that study are included in the present report. The data for this report are on 184 heifers from the Holstein-Friesian herd at the Emmons Blaine, Jr., Experimental Farm, Lake Mills, Wisconsin. This herd is composed of six sire lines and within each sire line there are four systems of mating practiced. These produce the following: outbred calves from outbred dams mated to outbred sires of another line (O-O matings); outbred calves from inbred dams mated to inbred sires of another line (O-I matings); inbred calves from outbred dams mated to related sires (I-O matings); and inbred calves from inbred dams mated to related sires (I-I matings). Coefficients of inbreeding range from 0.16 to 0.39 for the inbred heifers. The study includes heifers which were 1 yr. of age after December 20, 1949, and before December 21, 1955. Animals in this herd are drylot-fed from birth until they leave the herd. Heat checks are made twice daily on heifers over 6 mo. of age. Puberty is defined as the first observed estrus. The heifers are bred at the first estrus after 15 mo. of age, providing that their reproductive organs are in breeding condition, as determined by rectal palpation following the procedure outlined by Buch *et al.* (1).

The data include the occurrence of calfhood scouring, weight at 6 mo. of age, weight gain between 6 and 12 mo. of age, weight at three to seven days after the first calving, age at puberty, and the milk production (M.E.) and the average butterfat percentage for the first 90 days following the first calving of a heifer. Calfhood scouring as an all-or-none trait is considered to have occurred if the heifer scoured for three or more consecutive days before 6 mo. of age. The average butterfat percentage is the average of the first three monthly five-day composite tests of the lactation.

The seasons are designated as follows: winter, December 21–March 20; spring, March 21–June 20; summer, June 21–September 20; and fall, September 21–December 20.

The data were analyzed by the method of fitting constants, as described by Kempthorne (11). This technique provides unbiased estimates of the over-all mean and the various subclass means. Sire lines were considered as a random variable, being fairly representative of the Holstein population; whereas, season

TABLE 1

Mean ages at puberty (days) classified by sire lines and systems of mating

Sire line	System of mating[a]				Sire-line average
	O-O	O-I	I-O	I-I	
1	354 (8)[b]	322 (2)	402 (13)	401 (9)	370 (32)
2	361 (9)	364 (1)	377 (14)	365 (5)	367 (29)
3	331 (11)	305 (5)	344 (12)	293 (3)	318 (31)
4	339 (11)	297 (2)	346 (13)	274 (1)	314 (27)
5	372 (17)	273 (4)	358 (13)	400 (3)	351 (37)
6	349 (10)	345 (5)	395 (9)	314 (4)	351 (28)
System of mating av.	351 (66)	318 (19)	370 (74)	341 (25)	345 (184)

[a] O-O, outbred heifers from outbred dams. O-I, outbred heifers from inbred dams. I-O, inbred heifers from outbred dams. I-I, inbred heifers from inbred dams.
[b] Denotes number of animals in the group.

of birth and system of mating were considered as fixed variables. The various cause-and-effect relationships were determined by the sequential order of their appearance in an animal's life.

RESULTS AND DISCUSSION

The mean ages at puberty for the various sire line and system of mating subclasses and also the numbers of animals represented are given in Table 1. The over-all mean age at puberty was 345 days, the median was 380 days, and the puberal ages ranged from 210 to 550 days. Assuming that the O-O group is fairly representative of outbred Holstein heifers, a comparison of their puberal age to reports by other workers is in order. The average age at puberty of these heifers was 351 days, which is slightly longer than the 11 mo. reported by Eckles (4) or the 334 days found by Donald and Anderson (3) for Holstein heifers. This age is in close agreement with the 11.2 mo. of age at puberty reported by Reid *et al.* (12) for Holstein heifers fed a normal level of nutrition.

The correlations and regressions, obtained by pooling the sums of squares and cross-products within subclasses, involving the variables calfhood scours, 6-mo. weight, 6- to 12-mo. weight gain, and age at puberty are given in Table 2. The regressions were tested for heterogeneity by the method described by Snedecor (15). Calfhood scouring was correlated ($r = -0.42$, $P < 0.01$) with 6-mo. weight and caused an average decrease of 38 lb. when it occurred. Scouring was not significantly associated with weight gain between 6 and 12 mo. of age. The regressions between scouring and age at puberty were heterogeneous for the various seasons of birth. Correlations between these two variables within the various seasons revealed that only in the fall-born animals was this association significant ($r = -0.78$, $P < 0.01$). Scouring in these fall-born animals delayed puberty 135.6 days. The correlation of 6-mo. weight with 6- to 12-mo. weight gain was not significant. Both 6-mo. and 6- to 12-mo. weight gain were negatively associated with age at puberty and had the following respective correlations, -0.57 ($P < 0.01$) and -0.22 ($P < 0.05$). The regressions indicate that age at puberty was decreased 0.77 and 0.36 days, respectively, for each additional pound of weight at 6 mo. of age and gain in weight between 6 and

TABLE 2

Interrelationships involving calfhood scours, 6-mo. weight, 6- to 12-mo. weight gain, and age at puberty in 156 heifers

Dependent variable	Independent variable	Correlation	Linear regression
6-mo. weight	Scours	−0.42**	−38.00 lb.[a]
6- to 12-mo. wt. gain	Scours	0.07	5.20 lb.[a]
Age at puberty	Scours		H
6- to 12-mo. wt. gain	6-mo. weight	0.12	0.10 lb.[b]
Age at puberty	6-mo. weight	−0.57**	−0.77 day[b]
Age at puberty	6- to 12-mo. wt. gain	−0.22*	−0.36 day[b]
Age at puberty	6-mo. weight (sire lines)	−0.82*	−1.08 day[b]
Age at puberty	6-mo. weight (system of mating)	−0.79	−0.87 day[b]

* $P < 0.05$.
** $P < 0.01$
H–Heterogeneous among seasons of birth.
[a] Per occurrence.
[b] Per pound.

12 mo. of age. The correlations of age at puberty with 6-mo. weight among the sire-line means (−0.82, $P < 0.05$) and the system-of-mating means (−0.79) agree very closely with the correlation of these two variables between individuals (−0.57). This close agreement tends to indicate a similar dependence of age at puberty on 6-mo. weight, whether variation in the latter is brought about by individuality, age, or sire-line differences.

The standard partial regression coefficients of age at puberty on calfhood scouring and 6-mo. weight in the fall-born animals were, respectively, 0.44 and −0.48 (Table 3). These two coefficients differed significantly from zero and from each other. This indicates that scouring caused a delay in puberty which could not be accounted for by its effect through 6-mo. weight alone in these fall-born heifers. In these heifers, scouring and 6-mo. weight accounted for 72% of the variation in age at puberty. Using all the heifers, the standard partial regression coefficients of age at puberty on 6-mo. and 6- to 12-mo. weight gain were −0.55 ($P < 0.01$) and −0.15 ($P < 0.05$), respectively. These two coefficients also differed significantly from each other. Thus, it appears that both periods of growth are important in determining age at puberty, with the early growth, however, being more important. Approximately 35% of the variation in age at puberty is accounted for by these two independent variables. These

TABLE 3

Standard partial regression coefficients of age at puberty on certain variables

Independent variable	Standard partial regression
Calfhood scours	0.44**[a]
6-mo. weight	−0.48**[a]
6-mo. weight	−0.55**
6- to 12-mo. weight gain	−0.15*

* $P < 0.05$
** $P < 0.01$.
[a] Fall-born heifers only.

TABLE 4

Analysis of variance of age at puberty

Source	DF	Error term	Mean square	
			Unadjusted ages	Adjusted ages[a]
(a) Season of birth	3	e	45	81
(b) System of mating	3	d	132*	40
(c) Sire line	5	f	88*	42
(d) Line × system	15	f	37	27
(e) Residual interaction	46		28[b]	42[b]
(f) Within subclass	111		32	23[c]

* $P < 0.05$.

[a] Individual ages adjusted for 6-mo. weight.

[b] Residual interaction mean square is composed of the mean squares of all the possible interactions involving season of birth.

[c] 110 DF; 1 DF lost by adjustment of age at puberty for 6-mo. weight.

results agree with observations made by many workers that the faster-growing animals reach puberty earlier. However, the results do not indicate whether somatic growth influences age at puberty per se or if they are both genetically and environmentally controlled by another factor.

The analysis of variance (Table 4) revealed that sire line and system of mating affected significantly the age at which an animal attained puberty. Season of birth and the interaction of sire line with system of mating were not significant. Sire lines 3 and 4, with respective mean puberal ages of 318 and 314 days, differed ($P < 0.05$) from the four other lines. At the 0.01 level of probability, Sire lines 3 and 4 differed only from Lines 1 and 2 which had mean puberal ages of 367 and 370 days, respectively. Among the systems of mating, the line-cross heifers (O-I), 318 days, differed ($P < 0.01$) from only the I-O group, 370 days in age at puberty. The I-I and the O-O heifers did not differ from either the I-O or the O-I groups.

The analysis of variance of 6-mo. weight indicated that sire line and system of mating had significant effects. This information plus that of the similar associations between lines, systems, and individuals for age at puberty with 6-mo. weight suggested that any effect of sire line and system of mating on age at puberty may be through their effect on 6-mo. weight. Individual ages at puberty were then adjusted for 6-mo. weight by using the over-all regression value of -0.77 between these two variables. An analysis of variance of the adjusted puberal ages (Table 4) revealed that the statistical significance of the effects of sire line and system of mating was removed. The difference in age at puberty of 56 days between the extreme lines was reduced to 40 days. The O-I heifers had an adjusted age at puberty of 338 days and the I-O group, 357 days; thus, the previous difference of 52 days between these two groups was reduced to 19 days after adjusting for 6-mo. weight.

The O-I heifers, which reached puberty the earliest, had the lowest incidence of scours, 1.5%, and were the heaviest in weight at 6 mo. of age, 407 lb. The I-O heifers with the latest puberal age had a high incidence of scouring, 43%, and were the lightest in weight at 6 mo. of age, 365 lb. Thus, it would appear

that heterosis may be showing up in the line-crosses and an inbreeding depression in the I-O system of mating. Foote *et al.* (6), in swine, considered the earlier puberal ages of line-cross gilts compared to inbred gilts as due to heterosis.

Season of birth was found to affect age at puberty adjusted for 6-mo. weight in the nonscouring group of 49 O-O heifers. The spring-born heifers differed ($P < 0.05$) from the winter-born heifers in age at puberty, 328 days compared to 390 days. This observation differs from the reports in swine, rabbits, and birds, in which spring-born animals were the latest to attain sexual maturity.

To determine whether the rate of sexual development has a role in determining the production of a heifer in her first lactation, the interrelationships among age at puberty, 6-mo. weight, calving weight, 90-day milk production (M.E.), and the average 90-day butterfat percentage were calculated on 136 heifers (Table 5). The variables were adjusted to remove the effects of calfhood

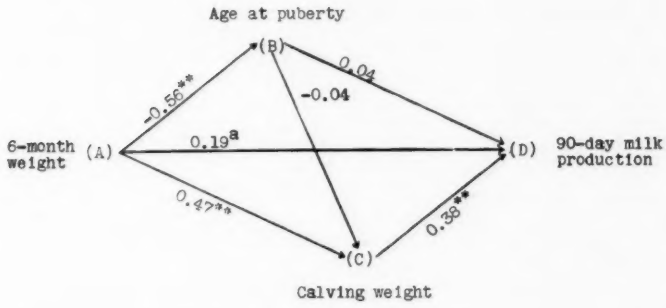
TABLE 5

Interrelationships involving age at puberty, 6-mo. weight, calving weight, 90-day milk production, and average 90-day butterfat percentage in 136 heifers[a]

Dependent variable	Independent variable	Correlation	Linear regression
Age at puberty	6-mo. weight	−0.56**	−0.77 days[b]
Calving weight	6-mo. weight	0.50**	1.20 lb.[b]
Calving weight	Age at puberty	−0.31**	−0.48 lb.[c]
90-day milk	6-mo. weight	0.35**	6.33 lb.[b]
90-day milk	Age at puberty	−0.18*	−2.57 lb.[c]
90-day milk	Calving weight	0.45**	4.02 lb.[b]
Butterfat (%)	6-mo. weight		H
Butterfat (%)	Age at puberty	−0.31**	−0.019%[c]
Butterfat (%)	Calving weight	0.28**	0.019%[b]

* $P < 0.05$.
** $P < 0.01$.
H—Heterogenous among sire lines.
[a] Variables adjusted for calfhood scouring.
[b] Per pound.
[c] Per day.

scouring. The 90-day milk production had the following respective correlations with age at puberty, 6-mo. weight, and calving weight, −0.18 ($P < 0.05$), 0.35 ($P < 0.01$), and 0.45 ($P < 0.01$). The regressions indicated that milk was increased 2.57 lb. for every day earlier in age at puberty, 6.33 and 4.02 lb. for every additional pound of weight at 6 mo. of age and at calving, respectively. Age at puberty and calving weight had highly significant correlations with the average 90-day butterfat percentage, −0.31 and 0.28, respectively. The butterfat percentage was increased 0.019% for every day earlier in age at puberty and also for every additional pound in weight at calving. The regressions among the sire lines of butterfat percentage on 6-mo. weight were heterogeneous. The correlations between these two variables ranged from −0.14 to 0.47 among the lines. Calving weight was significantly correlated with 6-mo. weight, 0.50 ($P < 0.01$), and age at puberty, −0.31 ($P < 0.01$).

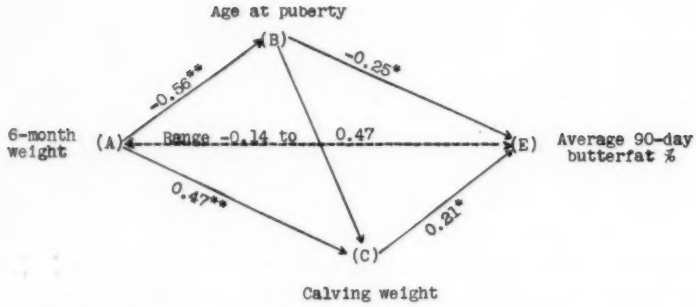


** $P < 0.01$.
* $P < 0.05$.
a $P < 0.10 > 0.05$.

FIG. 1. Path diagram with standard partial regression coefficients of the various relationships involving 90-day milk production.

Figure 1 is the path diagram with the standard partial regression coefficients of the various cause-and-effect relationships concerning 90-day milk production. The standard partial regression of age at puberty on 6-mo. weight was −0.56, those for calving weight on 6-mo. weight and age at puberty were 0.47 and −0.04, respectively, with the first two being significant ($P < 0.01$). The path coefficients of 90-day milk production on age at puberty, 6-mo. weight, and calving weight were 0.04, 0.19 ($P < 0.10 > 0.05$), and 0.38 ($P < 0.01$), respectively. The multiple correlation of the different variables with milk production was 0.48 and they accounted for 23% of the variation in the milk production. Thus, it appears that age at puberty does not have any significant role in determining milk production independent of 6-mo. and calving weight. The weight at 6 mo. of age appears to exert some effect independent of that which is expressed through calving weight. Calving weight had the most important effect on milk production of the variables studied. The various pathways and their relative importance by which 6-mo. weight is capable of influencing the 90-day milk production were: directly (AD), 0.19; through calving weight (ACD), 0.18; through age at puberty (ABD), −0.02, and through age at puberty and calving weight (ABCD), 0.01. From this, it appears that there are two relatively equal and important routes by which 6-mo. weight may effect the first 90-day milk production of a heifer's lactation, these being directly and through calving weight.

Figure 2 shows the relationships of the variables with the average 90-day butterfat percentage. The relationships involving 6-mo. weight, age at puberty, and calving weight have the same values as in Figure 1. A meaningful standard partial regression coefficient of butterfat percentage on 6-mo. weight could not be obtained because of the heterogeneity existing between them. The path coefficients of butterfat percentage on age at puberty and calving weight were −0.25 and 0.21, respectively, each differing ($P < 0.05$) from zero and from the other.



** $P < 0.01$.
* $P < 0.05$.

FIG. 2. Path diagram with standard partial regression coefficients of the various relationships involving the average 90-day butterfat percentage.

The different variables had a multiple correlation of 0.37 with the average 90-day butterfat percentage, and accounted for 14% of its variation.

Whereas, age at puberty apparently does not have an important role independent of growth rate in determining the amount of milk produced in the first 90 days of a heifer's lactation, it does have a significant direct role in determining the average butterfat percentage in the milk. Calving weight also had a significant role in determining the butterfat percentage. The pathways, other than the direct one, by which 6-mo. weight may affect the average butterfat percentage were: through age at puberty (ABE), 0.14; through calving weight (ACE), 0.10; and through age at puberty and calving weight (ABCE), 0. It appears from these results that 6-mo. weight may be exerting slightly more influence on the average butterfat percentage through age at puberty than through calving weight. In this group of heifers those which weighed the most at 6 mo. of age and had the earliest ages at puberty had the highest milk production, with the highest average butterfat percentage for the first 90 days following calving.

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ENVIRONMENTAL INFLUENCES ON REGRESSION FACTORS FOR ESTIMATING 305-DAY PRODUCTION FROM PART LACTATIONS[1]

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SUMMARY

Cumulative milk and fat production on test days from 11,420 Michigan DHIA-IBM records from 1953 to 1957 were used to derive regression factors for estimating 305-day production from part lactations. The data included 8,993 Holstein, 1,457 Guernsey, 651 Jersey, and 319 Brown Swiss lactations.

Linear regression coefficients of total on part lactation within herd-season-lactation-age groups were used to measure the relationship between cumulative production on test day and total production for the first ten test days. Analyses of regression coefficients for the first, third, and seventh cumulative months of the Holstein data indicated season was not an important influence on the part-to-whole relationship. Visual inspection of the mean coefficients for age within lactation numbers failed to reveal where ages and parities should be separated or combined.

Intra-herd factors for extending incomplete records were developed from the average regression coefficients of total on part lactations within herds, seasons, lactations, and ages for each of the nine cumulative test days. Each regression coefficient included in the average was weighted by the number of records from which it was computed. For comparison, extension factors disregarding herd, season, lactation number, and age effects also were derived. Although the differences between inter- and intra-herd factors were not tested for significance, the marked similarity between the Holstein factors suggests that herd had little or no influence on the part-to-whole relationship.

Correlations between cumulative part and 305-day production ignoring herd differences were not less than 0.7 for the first month, increased steadily as the lactation progressed, and were 0.9 by the fifth test day for all breeds. These findings support data in the literature which suggest that production records of only 1 or 2 mo. are valuable guides to what a cow will produce in that lactation. Estimating 305-day production from such records would be useful in early culling and progeny testing.

Approximately half of the tested cows in Michigan leaving the milking herd are removed because of low production (11). Many of those removed are young cows with less than a complete 305-day lactation. Excluding their incomplete records from sire proofs may distort the results to make some sires appear better than they would if all progeny records were included.

If 305-day production could be estimated from parts of the lactation with reasonable accuracy, partial lactation records could be included in sire proofs. This would help to eliminate selective progeny testing resulting from the removal of low-producing cows before they can complete their first record of production. Estimates from part records also could provide an early evaluation of bulls and their daughters before the daughters have completed their first records. Culling decisions on cows early in their first or second lactation would be possible.

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Ratio and regression factors have been developed to estimate 305- or 365-day lactation yield from a partial record or production tests made during certain portions of the lactation (2, 3). Gifford (4) reported that each month was more closely correlated with the production of the months in the same lactation than with the production for the same month in a subsequent lactation. Large correlations between the cumulative production for the first few months and the complete lactation yield have been reported (7, 12).

Previous work has indicated that breed, age, season of calving, milking frequency, and stage of gestation influence the quantity of milk and fat produced during all or portions of the lactation. How much influence these factors exert on the relationship between a partial record and its 305-day equivalent has not been evaluated completely. Recently, workers have studied the differences between extension factors computed for different seasons, ages at calving, and milking frequencies (5, 6, 8, 9, 10).

This study was initiated to examine the importance of breed, herd, lactation number, season, and age at calving on the relationship between milk and fat production on test day and production for the complete lactation; and to compute regression factors for estimating 305-day production from part lactations.

EXPERIMENTAL PROCEDURE

Production on monthly test day in Michigan DHIA-IBM from May, 1953, to July, 1957, was recorded to the nearest tenth of a pound of milk and to the nearest hundredth of a pound of fat for each cow. Lactations having less than ten consecutive test days were eliminated from the study. Lactations having more than 49 days elapsing between the date of calving and the first test were excluded to remove the possibility of having ten consecutive tests during a lactation in which the cow was not tested the first month after calving. The numbers of records used in the analyses were 12,562 Holstein, 2,262 Guernsey, 990 Jersey, and 459 Brown Swiss. Cumulative test-day production for each lactation was obtained by adding consecutively the production on each test day to the sum of the yields from previous test days.

In order to study postulated environmental influences on the part-to-whole relationship of lactations, the data were grouped by two 6-mo. seasons of calving, three lactation numbers, and eight ages of calving. The seasons of calving corresponded to pasture and barn seasons, October to March and April to September. Lactation groups were first, second, and all subsequent lactations. The age groups were divided by 6-mo. intervals, but all records initiated at less than 24 mo. were in a single group, and all 60 mo. or over, in another.

Whether the partial records of cows freshening for the second time can be extended accurately, if they are treated the same as those freshening for the first time at the same age, has not been examined previously. The choice of groupings was made to facilitate determining the significance of early and late calving on the part-to-whole relationship and was guided by the relationship in Figures 1 and 2.

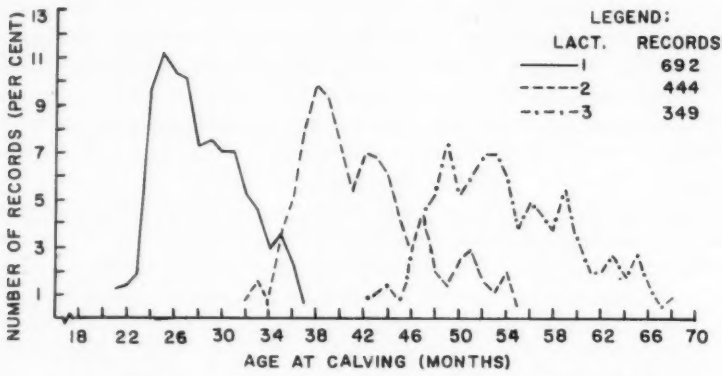

FIG. 1. Frequency of Guernsey records by age and lactation number.

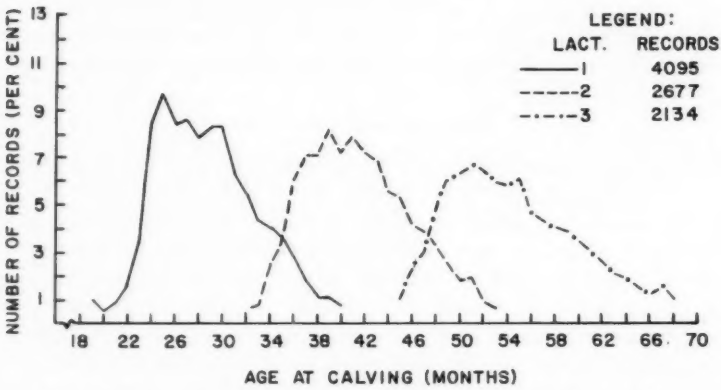

FIG. 2. Frequency of Holstein records by age and lactation number.

The numbers of first, second, and third lactations of the Guernseys and Holsteins are plotted separately by age at calving in Figures 1 and 2. The first lactation curve intercepts the second lactation curve at 35 mo. of age. The second lactation curve intercepts the third at 47-48 mo. Similar characteristics were observed by Madden *et al.* (10) with Holstein data.

Linear regression coefficients of total on part lactation within each herd-season-lactation-age group were calculated to the nearest hundredth of a pound for each of the nine cumulative test days. These regression coefficients were used as the variable measuring the relationship between production for the cumulative part and the complete ten test days. Variation between the intra-herd coefficients for the various season-lactation number-age groupings should reflect the significance of the environmental agents under consideration.

Although a more cumbersome statistic with which to work, regression factors were used instead of ratios to develop factors which would be appropriate if

level of production of the cow were to be included in the adjustment of part to whole. At least two observations were necessary to fit a regression line to each intra-herd-season-lactation-age group; therefore, all records occurring singly in a class were eliminated from the study. The numbers of usable records remaining were 8,993 Holstein, 1,457 Guernsey, 651 Jersey, and 319 Brown Swiss.

Analyses of variance and visual inspection of milk and fat regression coefficients for the first, third, and seventh cumulative months of the Holstein records were used to determine the importance of season, lactation number, and age on the part-to-whole relationship.

Factors for estimating 305-day yield from part lactations were computed as the average regression coefficient for each cumulative test day within all herd-season-lactation-age groups. Each coefficient in the average was weighted by the number of records included. For comparison, inter-herd extension factors ignoring herd, season, lactation number, and age differences were computed.

RESULTS AND DISCUSSION

Season. The structure of the analysis of variance to determine the importance of season on the relationship between the part and complete lactation is presented in Table 1. The significance of the analysis is weakened by all

TABLE 1

Analysis of variation in regression coefficients for herds and seasons—Holstein

Test day	Source	DF	Milk	Fat
			Mean square	Mean square
	Total	2,698		
	Between seasons	1	476	986*
1	Between herds	634	1,413**	255
	Herd × season[a]	421	1,005	253**
	Residual	1,642	1,208	215
	Total	2,698		
	Between seasons	1	0	21
3	Between herds	634	98	60
	Herd × season[a]	421	77	59
	Residual	1,642	114	80
	Total	2,698		
	Between seasons	1	30	49
7	Between herds	634	25	11
	Herd × season[a]	421	39**	16**
	Residual	1,642	13	12

* $P < .05$. ** $P < .01$. Due to disproportionality, these are approximations.

[a] Ninety-four herds had records in the October-March season exclusively; 119 herds had records in the April-September season exclusively.

herds not having proportional numbers of cows freshening in both seasons. Consequently, if the mean square for interaction (herd × season) is used to test the significance of the variation attributed to herds and seasons, the probabilities will be inexact and indicate trends rather than precise differences. An analysis of a proportional group of observations would be more conclusive in answering the question of seasonal influence on the part-to-whole relationship, but these do not occur.

Variation due to season was significant only for the first test day of fat production. Significant herd differences were noted for the first test day of milk production. Herd × season interactions were significant for the first test day of fat and for the seven cumulative test days of milk and fat. Differences in fat production due to season, especially for the first test day, would be expected, since a direct relationship of season of the year and per cent of butterfat has been found regardless of the stage of lactation (1, 14). The lowest fat test usually occurs during June-July and the peak test in December-January. Because the smallest percentage of incomplete records is terminated in the first month of the lactation (13), and the seasonal effect for the first month is expected, season was not considered an important environmental factor influencing the relationship between the cumulative part and whole lactation.

Lactation number and age. Unweighted average regression coefficients of total on part lactation for cows of the same age in the same lactation for the

TABLE 2

Average regression coefficients for ages and lactation groups—Holstein

Test day	Lactation group	Age group *(months)*							
		−24	24–29	30–35	36–41	42–47	48–53	54–59	60+
		(No. of coefficients)							
	1	53	493	318	67	12	....	....	....
	2	....	1	33	284	206	48	9	4
	3	....	....	1	5	27	193	176	769
		(Pounds of milk)							
	1	5.55	6.63	6.33	4.05	6.89	....	....	....
1	2	....	−.61	5.35	4.90	4.17	6.46	14.62	−79.44
	3	....	....	27.54	20.98	4.20	6.27	4.82	4.75
	1	−.49	1.89	2.93	2.56	2.60	....	....	....
3	2	....	−.56	3.47	2.34	2.27	1.51	2.04	4.09
	3	....	....	2.13	4.57	4.93	1.60	2.64	1.85
	1	1.40	1.49	1.35	1.19	1.43	....	....	....
7	2	....	2.59	1.09	1.15	1.43	.89	.11	1.50
	3	....	....	.84	1.48	1.77	1.19	1.12	1.48
		(Pounds of fat)							
	1	5.42	3.29	3.65	8.36	3.26	....	....	....
1	2	....	.41	7.88	2.83	3.50	5.20	−1.04	1.12
	3	....	....	12.25	−6.64	13.84	1.10	3.92	2.69
	1	1.84	1.98	2.53	2.64	2.23	....	....	....
3	2	....	.61	3.79	1.55	1.14	−1.18	1.79	4.77
	3	....	....	2.01	−2.16	1.97	2.33	2.29	1.63
	1	1.18	1.37	1.21	1.25	.73	....	....	....
7	2	....	−11.50	.84	1.92	1.41	1.24	1.56	1.72
	3	....	....	1.23	−2.85	−1.56	1.34	.97	1.27

Holstein data are presented in Table 2. No distinct pattern was observed where ages differed within or between lactation groups. The numbers of coefficients determining averages for the 30-35 and 36-41 mo. age groups of the first and second lactations were the most nearly proportional. Yet, the relationship be-

tween the age coefficients for a given month was not consistent for the three test days analyzed.

A comparison of records initiated before and after 36 mo. of age for the first and second lactations showed less overlapping of the two lactations than previously reported (10). Only 7% of the first lactation records were initiated after 36 mo. and 5% prior to 36 mo. for the second lactations, as compared to 12 and 10%, respectively, reported by Madden *et al.* (10). Based on these findings, age at calving and lactation number were ignored in the development of factors for the part-to-whole relationship.

Extrapolation factors. Extension factors based on cumulative production would be expected systematically to approach unity as the lactation progressed. The average regression factors computed did not exhibit this characteristic, except for the Holsteins. The observed fluctuation from one test day to the next could possibly be explained by the use of intra-herd regression coefficients as a measure of relationship and the relatively small numbers of records controlling their value. Regression coefficients produced by the minimum of only two records falling in a single herd-season-lactation-age group could easily change the differences between each other in their part-to-whole relationship from one test day to the next. Consequently, if there were sufficient numbers of coefficients of this nature, the changing relationship would be reflected in the unweighted averages.

TABLE 3

Regression factors for extending cumulative test-day production to a 305-day basis—Holstein*

Test day	A	a	B	b
		(Pounds of milk)		
1	50.4	12.3	5.09	5.19
2	100.4	23.8	2.91	2.62
3	146.4	33.4	2.11	2.15
4	188.9	43.8	1.72	1.69
5	228.5	52.3	1.47	1.61
6	265.5	60.0	1.31	1.37
7	300.3	67.0	1.20	1.31
8	332.5	73.0	1.11	1.19
9	361.2	78.1	1.05	1.11
10	384.7	82.0	1.00	1.00
		(Pounds of fat)		
1	1.95	.59	4.04	3.32
2	3.70	1.00	2.54	2.42
3	5.30	1.36	1.96	1.89
4	6.78	1.64	1.64	1.77
5	8.17	1.96	1.44	1.51
6	9.49	2.22	1.30	1.43
7	10.73	2.46	1.19	1.28
8	11.91	2.67	1.11	1.15
9	12.98	2.86	1.05	1.08
10	13.88	3.00	1.00	1.00

* 8,993 lactations, 2,699 regression coefficients.

A is cumulative test-day production. a is the standard deviation of cumulative test-day production. B is the inter-herd regression of total on cumulative part production. b is the intra-herd regression of total on cumulative part production.

When the data were scrutinized, 45-55% of the regression coefficients were the products of two observations. The numbers of intra-herd-season-lactation-age regression coefficients of total on part lactation for the four breeds were: 2,699 Holstein, 440 Guernsey, 208 Jersey, and 98 Brown Swiss. The number of regressions might seem sufficient to average out much of the random variation among the coefficients. The results indicate, however, that variation among the coefficients was so large and the number of regressions per subclass so small that sampling variation caused the average coefficients to fluctuate widely.

When each intra-herd-season-lactation-age coefficient was weighted by the number of records controlling its value, the trends in the average regression coefficients were smoothed out to a considerable extent. By weighting in this manner, the average regression coefficients were determined more by the regressions fitted to many records within a single herd-season-lactation-age group. The corresponding coefficients have less tendency to deviate excessively from one test day to the next. The weighted extension factors (b) are presented for the various breeds in Tables 3-6.

Intra-herd extension factors (b) can be compared with inter-herd factors (B) in Table 3. Although the differences have not been tested for significance, a marked similarity exists between the two sets of factors, especially for milk production. Since variation due to herd differences is significant only for the first month of milk production (see Table 1), this suggests that herd differences

TABLE 4

Regression factors for extending cumulative test-day production to a 305-day basis—Guernsey*

Test day	A	a	B	b
		(Pounds of milk)		
1	37.1	8.5	4.79	3.30
2	72.8	16.4	2.79	2.05
3	105.0	23.5	2.05	2.08
4	134.2	30.0	1.67	2.01
5	161.0	35.8	1.45	1.42
6	185.9	41.0	1.30	1.01
7	209.3	45.7	1.19	1.30
8	231.0	49.8	1.12	1.19
9	250.4	53.3	1.06	1.08
10	266.7	56.7	1.00	1.00
		(Pounds of fat)		
1	1.72	.46	3.97	2.97
2	3.32	.83	2.51	2.12
3	4.79	1.13	1.96	2.54
4	6.16	1.41	1.65	1.63
5	7.44	1.66	1.45	1.77
6	8.66	1.88	1.31	1.20
7	9.80	2.10	1.21	1.16
8	10.90	2.29	1.13	1.20
9	11.88	2.47	1.06	1.11
10	12.73	2.64	1.00	1.00

* 1,457 lactions, 440 regression coefficients.

A is cumulative test-day production. a is the standard deviation of cumulative test-day production. B is the inter-herd regression of total on cumulative part production. b is the intra-herd regression of total on cumulative part production.

TABLE 5

Regression factors for extending cumulative test-day production to a 305-day basis—Jersey*

Test day	A	a	B	b
		(Pounds of milk)		
1	32.6	7.7	4.75	3.51
2	64.0	14.5	2.80	1.98
3	92.1	20.6	2.09	2.07
4	117.3	26.3	1.71	1.99
5	140.3	31.5	1.48	1.37
6	161.9	35.8	1.32	1.29
7	182.1	40.2	1.20	1.01
8	201.0	43.9	1.12	1.13
9	218.0	47.1	1.06	1.05
10	232.7	50.0	1.00	1.00
		(Pounds of fat)		
1	1.60	.46	4.02	3.18
2	3.14	.80	2.62	2.65
3	4.57	1.09	2.04	1.74
4	5.88	1.36	1.69	1.70
5	7.11	1.61	1.48	2.34
6	8.27	1.85	1.32	1.80
7	9.36	2.07	1.21	1.48
8	10.40	2.25	1.12	1.27
9	11.34	2.42	1.06	1.12
10	12.17	2.58	1.00	1.00

* 651 lactations, 208 regression coefficients.

A is cumulative test-day production. a is the standard deviation of cumulative test-day production. B is the inter-herd regression of total on cumulative part production. b is the intra-herd regression of total on cumulative part production.

TABLE 6

Regression factors for extending cumulative test-day production to a 305-day basis—Brown Swiss*

Test day	A	a	B	b
		(Pounds of milk)		
1	42.9	11.2	5.11	4.03
2	85.8	22.2	2.80	3.47
3	125.9	31.7	2.07	1.66
4	163.2	40.3	1.69	1.37
5	197.8	48.1	1.46	1.60
6	230.3	55.1	1.30	1.28
7	260.5	61.3	1.19	1.29
8	288.2	66.4	1.12	1.19
9	313.1	70.9	1.06	1.07
10	334.2	75.2	1.00	1.00
		(Pounds of fat)		
1	1.72	.53	4.40	3.25
2	3.34	.93	2.72	2.66
3	4.86	1.29	2.08	1.76
4	6.29	1.61	1.73	1.73
5	7.63	1.91	1.50	1.52
6	8.91	2.18	1.33	1.27
7	10.12	2.43	1.22	1.19
8	11.25	2.66	1.13	.97
9	12.28	2.85	1.06	1.14
10	13.19	3.04	1.00	1.00

* 319 lactations, 98 regression coefficients.

A is cumulative test-day production. a is the standard deviation of cumulative test-day production. B is the inter-herd regression of total on cumulative part production. b is the intra-herd regression of total on cumulative part production.

are not an important factor influencing the part-to-whole relationship of lactations. Consequently, it may not be necessary or practical to derive extension factors on an intra-herd basis to achieve maximum accuracy in extending incomplete records to a 305-day basis.

Means (A) and standard deviations (a) of cumulative test day production averaged over all herds also are presented. Differences between herds and cows are included in these values. The average variation was less for the smaller breeds (Guernsey and Jersey) than for the larger breeds (Holstein and Brown Swiss). The variation in cumulative production increased at a decreasing rate for all breeds.

Correlations. Correlations (r) between cumulative test day production and the sum of the ten consecutive test days ignoring herd differences are presented in Table 7. They compare quite closely with those reported in the literature.

TABLE 7

Correlations between cumulative test-day and total production, and standard errors of estimate*

Test day	Holstein		Brown Swiss		Guernsey		Jersey	
	r	e	r	e	r	e	r	e
			(Pounds of milk)					
1	.76	61.1	.76	57.1	.72	44.1	.73	38.6
2	.85	48.9	.82	48.9	.81	36.2	.81	31.5
3	.88	42.1	.87	41.2	.85	31.6	.86	26.9
4	.92	35.3	.91	35.2	.89	27.8	.89	23.7
5	.94	30.9	.93	29.8	.91	24.1	.92	20.3
6	.96	25.7	.95	24.6	.94	20.3	.94	17.0
7	.98	18.6	.97	19.1	.96	16.1	.97	13.5
8	.99	14.8	.99	13.3	.98	11.2	.98	9.6
9	1.00	7.1	1.00	7.2	.99	5.9	1.00	5.2
			(Pounds of fat)					
1	.77	2.6	.76	2.6	.70	2.4	.71	2.4
2	.84	2.0	.83	2.0	.79	2.0	.81	1.8
3	.89	1.6	.88	1.6	.84	1.6	.87	1.5
4	.92	1.4	.91	1.4	.88	1.4	.90	1.3
5	.94	1.2	.94	1.2	.91	1.2	.92	1.1
6	.96	1.0	.96	1.0	.94	1.0	.95	.9
7	.97	.8	.97	.7	.96	.8	.97	.7
8	.99	.5	.99	.5	.98	.5	.98	.5
9	1.00	.3	1.00	.3	.99	.3	.99	.3

* *r* is correlation ignoring herd, season, lactation, and age differences. *e* is standard error of estimate ignoring herd, season, lactation, and age differences.

The Holstein and Brown Swiss correlations compare very closely and are at least 0.9 by the fourth month. The Guernsey and Jersey are similar to each other and, although being lower in the initial stage of the lactation, are 0.9 by the fifth month.

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EFFECT OF CHLORPROMAZINE ON THE PERFORMANCE OF LACTATING DAIRY COWS

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SUMMARY

Studies were conducted to determine the oral dosage of chlorpromazine required to produce tranquilization in lactating Holstein cows and to evaluate the effects of feeding low levels of the tranquilizer on milk production during a 24-wk. period.

In a series of trials involving six cows, no tranquilization was observed when as much as 5 g. of chlorpromazine per day was divided into two equal portions and fed with the grain ration at the morning and evening feedings. On the other hand, two cows showed a marked degree of tranquilization after receiving single doses of 4.5 and 9.0 g. of the tranquilizer, respectively. Thus, approximately 5 mg. of chlorpromazine per pound of body weight was effective in producing tranquilization in lactating dairy cows. No chlorpromazine was detected in the milk from the cows at any level of administration.

Twenty-four additional cows were used to evaluate the effects of feeding three different levels of chlorpromazine, i.e., 0, 250, and 500 mg. per day, on milk production under different environmental conditions. No appreciable differences were observed among treatment groups with respect to the production of 4% fat-corrected milk or body weight changes during the 24 wk. of the experiment.

Tranquilizing agents have become widely used in veterinary practice during the last few years and some of them have shown promise of becoming useful feed adjuncts. Increased weight gains in beef calves and/or lambs due to the inclusion of certain tranquilizers in the ration have been reported by some workers (4, 14, 15); however, other reports (1, 5, 11) indicate no advantage attributable to these additives. Hoerlein and Marsh (6) observed that beef calves injected with chlorpromazine were less disturbed by weaning and made greater weight gains during the week following weaning than untreated controls. On the other hand, Henrickson *et al.* (5) found no increase in the weight gain of beef calves at weaning or during the 21- to 33-day period thereafter, following injection of either ethyl isobutrazine (Diquel) or trifluomeprazine.[1]

In discussing the various uses of tranquilizers in veterinary practice, Scheidy and McNally (12) stated that chlorpromazine is valuable in training first-calf heifers to stanchions and milking machines. Similarly, Jordan and Ward (7) reported that nervous first-calf heifers responded well to the milking routine after injections of a tranquilizer (Vetame[2]) for a period of several days postpartum.

Little information is available concerning the effect of tranquilizers on lactation. Meites (10) reported that intravenous injection of reserpine was

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[1] Smith, Kline and French designation for the compound, 10-(3-dimethyl-amino-2-methyl-propyl)-2-trifluoro-methyl-phenothiazine hydrochloride.

[2] Product of E. R. Squibb and Sons, New York, New York.

effective in initiating lactation in rabbits, although the lactation response was in no instance equal to that following parturition. Jordan and Ward (7) noted that injections of Vetame had no effect on milk production of mature, high-producing Holstein cows. Similarly, Lassiter *et al.* (8) reported that feeding 2.5, 5.0, 10.0, 30.0, or 50.0 mg. per day of hydroxyzine had no effect on milk production, fat test, body weight changes, or feed consumption of dairy cows.

Since no work had been reported on the effect of orally administered chlorpromazine on dairy cows, the present study was undertaken to obtain information concerning the performance of lactating cows fed this tranquilizer.

EXPERIMENTAL PROCEDURE

In the first phase of this study, graded levels of chlorpromazine hydrochloride were fed to lactating Holstein cows to determine the amount of the tranquilizer necessary to produce tranquilization. Five cows were used in a series of three four-day trials, during which levels of 0.05 to 5.0 g. of chlorpromazine were administered daily (Table 1). The daily allowance of tran-

TABLE 1

Levels of chlorpromazine administered orally to lactating Holstein cows

Period of administration	Cow No.					
	4	12	22	73	83	1
			(g/cow/day)[a]			
October 9-13	0.05	0.10	0.15	0.20	0.25	....
October 16-20	0.30	0.35	0.40	0.45	0.50	....
October 23-27	1.0	2.0	3.0	4.0	5.0	....
November 28	.....	.....	4.5	.....	.....	9.0

[a] Administered in two equal portions at morning and evening feedings, except for the last period, when the amounts were given as a single dose.

quilizer was divided into two equal portions and added to the grain ration at the time of the morning and evening feeding during these trials. An additional trial consisted of feeding two cows 4.5 and 9.0 g. of chlorpromazine, respectively, as a single dose.

The cows were observed each day for clinical evidence of tranquilization, and milk samples were collected on the last day of each of the four-day periods for chlorpromazine assay. From the animals receiving large single doses of tranquilizer, milk samples were obtained at approximately 7 and 19 hr. after administration. Samples of blood, rumen fluid, and urine were also taken for assay during some of the experimental periods. The method of Flanagan *et al.* (3) was modified to determine the total chlorpromazine and total chlorpromazine sulfoxide in these samples. This method permitted the detection of as little as 0.5 p.p.m. of chlorpromazine and 1.0 p.p.m. of chlorpromazine sulfoxide.

In another phase of this study, an additional 24 Holstein cows were used to evaluate the effects of feeding low levels of chlorpromazine on milk production under different environmental conditions. Throughout a 24-wk. period, three respective groups of eight cows each were fed 0, 250, and 500 mg. of chlorpromazine per cow daily. Clinical studies had shown that stress in cattle

due to shipping was controlled by parenteral administration of one- to two-tenths of the amount of chlorpromazine required to produce tranquilization (13); however, no information was available regarding the possible cumulative effects of this tranquilizer when administered orally over an extended period. Therefore, the levels of tranquilizer used in the present experiment were well below the level required to produce sedation in mature cows, with the daily intake of the higher-level group averaging 0.48 mg. of chlorpromazine per pound of body weight.

Since it was of interest to determine whether the tranquilizer might have a different effect on the production of cows under different environmental conditions, one-half of the animals were provided with an open-shed type of shelter while the other half had no shelter during the entire period from November 1 to April 17. Thus, after grouping on the basis of the time of freshening, the cows were randomly assigned to treatment groups according to a 2×3 factorial arrangement.

Following a period of liberal grain feeding, the cows were allotted grain at the beginning of the experiment according to Morrison's standard for cows not on pasture. Thereafter, the allowance for each cow was reduced each month based on the average decline in production of all the cows. The tranquilizer was premixed with cerelose and fed daily in two equal portions by adding it to the grain at the time of feeding. Alfalfa hay and sorghum silage were fed free-choice throughout the experiment.

Body weights were taken at the beginning of the experiment and every 2 mo. thereafter until its termination. Milk production was recorded twice daily and samples were taken weekly for butterfat tests throughout the 24 wk. of the experiment. Breeding of all the cows was delayed until the 2nd mo. after the experiment began, to minimize the effect of gestation on production.

The air temperature and humidity were determined by means of recording hygrothermographs and the miles of wind travel were recorded with Bendix-Friez, cup-type, totalizing anemometers.

RESULTS AND DISCUSSION

A relatively large oral dose of chlorpromazine was required to produce tranquilization in mature, lactating Holstein cows. No evidence of tranquilization was noted on the part of cows receiving as much as 5 g. per day of the tranquilizer in two equal portions for a four-day period (Table 1). However, two cows fed single doses of 4.5 and 9.0 g. of chlorpromazine, respectively, were observed to be tranquilized 2 hr. after administration and remained in this condition for several hours. Sedation was particularly noticeable on the part of the cow receiving the larger amount of the drug. This animal showed an absence of some common reflexes and did not resist various procedures, such as the insertion of a mouth speculum for taking a rumen sample. Similarly, Troughton *et al.* (16) reported that chlorpromazine-treated animals submit readily to various treatment procedures such as minor teat surgery, changing of bandages, examination of teeth, trimming of hooves, or insertion of nose rings.

In the present study, the chlorpromazine had no apparent effect on milk production, feed consumption, rumination, urination, or defecation of the cows during the period of tranquilization. However, the tranquilized cows had muscle tremors, chiefly affecting the rear quarters, following excitement due to the taking of blood samples. This adverse side effect, though temporary in nature, would likely limit the usefulness of this tranquilizer for some purposes.

The two cows showing some degree of tranquility in the present experiment received 3.9 and 9.5 mg. of chlorpromazine per pound of body weight, respectively. This is a considerably higher dosage range than the 1.0 to 1.5 mg. per pound level which has been suggested for other animals, such as dogs or cats (12). In this connection, Hoerlein and Marsh (6) reported that chlorpromazine injected at the rate of 1.25 mg. per kilogram of body weight was well tolerated by dairy calves, but caused incoordination in beef calves. Moreover, Henrickson *et al.* (5) observed a wide variation among beef animals in their response to injection of a given dose of either chlorpromazine, ethyl isobutrazine, or trifluomeprazine. The reasons for these differences among animals in their response to a given tranquilizer are not apparent.

No chlorpromazine or its major metabolite, chlorpromazine sulfoxide, was found in the milk or blood from the cows in this experiment even at the higher levels of administration. However, both compounds were present in the urine, and Henrickson *et al.* (5) estimated that 10 to 15% of injected chlorpromazine may be excreted via this route within 10 to 24 hr. after injection. Also, these workers found residues of the drug in certain organs of animals slaughtered 8 hr. or less following injection.

The production of 4% fat-corrected milk (FCM) was quite similar for the three groups of cows receiving different levels of chlorpromazine (Figure 1). The average production of the groups receiving 0, 250, and 500 mg. of tranquilizer per day was 4,524, 4,752, and 4,244 lb. of 4% FCM, respectively, for the 24-wk. period. The average gain in weight during this period was 90, 86, and 99 lb. for the three groups, respectively. An analysis of variance showed the differences among the three groups to be of no statistical significance ($P > 0.05$). Similarly, Lassiter *et al.* (8) reported that feeding hydroxyzine had no significant effect on milk production, whereas Jordan and Ward (7) noted no increase in production attributable to injections of the tranquilizer Vetame.

The average production of the cows provided with an open-shed shelter was 4,617 lb. of 4% FCM for the 24-wk. period, and the corresponding production for the animals having no shelter was 4,397 lb. of 4% FCM. The difference between these two groups was not statistically significant ($P > 0.05$), nor was there any significant interaction between the effects of the tranquilizer and shelter treatments in this experiment. The weather data showed that the outside air temperature was not greatly different from that within the open shed, although the animals with shelter were noticeably more comfortable during periods of low temperatures than were the cows in the other group. These observations support the opinion of Blaxter (2), that measurement of wind

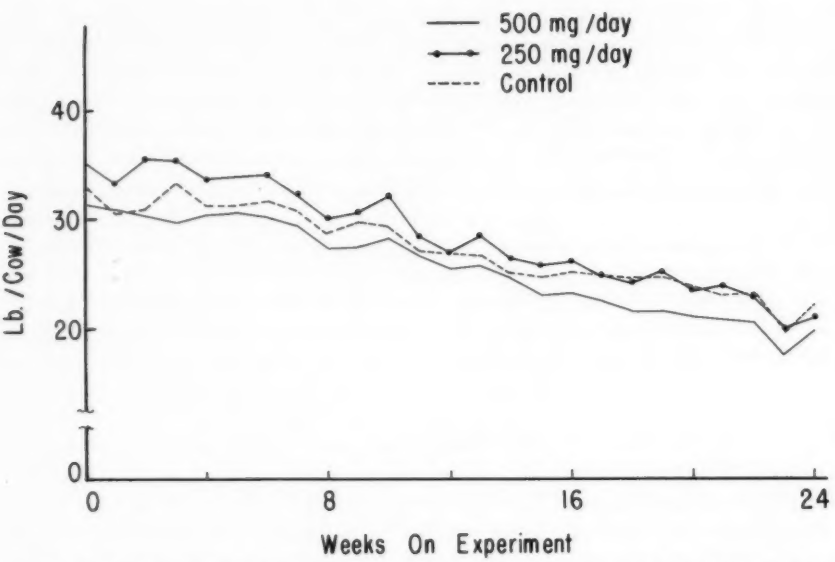

FIG. 1. Production of 4% fat-corrected milk by Holstein cows receiving different levels of chlorpromazine.

velocity and radiant temperature needs to be combined in some way with data on air temperature to give an adequate description of climate for farm animals. Nevertheless, the data obtained in the present experiment indicate there is little difference in the production of Holstein cows in an environment characterized by low, fluctuating temperatures, exposure to wind, and no protection from radiant cooling, as compared to an environment provided by an open-front shelter. Further, it appears that low, fluctuating temperatures within the range encountered in this experiment have no marked effect on the production of Holstein cows, provided that adequate feed and water are readily available. This view is supported by data of MacDonald and Bell (9) showing that low, fluctuating, ambient air temperatures as low as 0° F. cause only small changes in the heart rate, rectal temperature, and respiration rate of lactating Holstein cows.

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STUDIES OF MILK FEVER IN DAIRY COWS. VI. EFFECT OF THREE PREPARTAL DOSAGE LEVELS OF VITAMIN D ON MILK FEVER INCIDENCE

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SUMMARY

A field study involving 164 parturitions in Jersey cows, all with previous milk fever histories, in 26 Ohio herds was conducted to obtain further information on how much vitamin D must be fed prepartal to provide maximum protection against milk fever. The assigned vitamin D dosage, 15, 20, or 30 million units/day, was fed on a twice-a-day basis beginning approximately five days before the expected calving date and for one feed after parturition. If seven days feeding were completed before parturition, vitamin D feeding was terminated. On-schedule cows were those that received at least three days of vitamin D feeding and calved within one day after termination of vitamin D feeding. In all, there were 113 in the on-schedule group. In the off-schedule group ten cows calved too early and 41 calved too late. Three vitamin D concentrates were used: Viosterol (capsules), Type 142F Irradiated Dry Yeast, and Super Drex 3000.

The average per cent protection for the three dosages, based on 64% milk fever expectancy in Jersey cows with previous milk fever histories, was 67.1% for the 15 million unit level, 82.0% at the 20 million unit level, and 79.2% at the 30 million unit level. No statistically significant differences were found in milk fever incidence or per cent protection among the three vitamin D dosage levels. Vitamin D was fed for an average of 5.6 days (11.2 feedings) prepartal, including the day of parturition in the on-schedule group. Because the 15 million unit/day dosage level resulted in a lower average protection than the two other dosages, it was considered unwise to recommend less than 20 million units per day, until more numbers can be studied. There was no evidence, however, that 30 million units per day would be more effective than 20 million units per day. All three vitamin D concentrates used appeared to be equally effective when consumed. Certain cows did not accept irradiated yeast readily. The viosterol capsules had the advantages gained from forced feeding and Super Drex 3000 offered no palatability problem.

Evidence obtained indicated that protection increased for the first three days of vitamin D feeding, remained at a high level after the third through the seventh day, and dropped precipitously after one day had elapsed following cessation of vitamin D feeding.

In earlier reports (3, 4) data were presented on the effect of large doses of vitamin D, fed for three to eight days prepartal, on the blood serum level of calcium and phosphorus, and the incidence of parturient paresis (milk fever) in dairy cows. The maintenance of a high level of serum calcium and phosphorus during the critical postpartum period, along with lowered incidence, especially at the higher doses, indicated that vitamin D could be used effectively in the prevention of milk fever. Further evidence of the effectiveness of vitamin D in milk fever prevention has been published more recently by Laird (5) and Dell and Poulton (2).

In our experiments (3) numbers were too few to determine the minimum effective daily dose of vitamin D for maximum protection. Thus, in the spring of 1955 a field study was organized to obtain further information on how much vitamin D must be fed to provide maximum protection against milk fever.

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EXPERIMENTAL PROCEDURE

In this field study three dosage levels of vitamin D were fed daily, 15, 20, or 30 million units. One-half the daily dose was fed in the morning and the other half in the evening.

Three different vitamin D concentrates were used during the period of the experiment; (1) Viosterol (irradiated ergosterol in oil, one million units per gram, administered in gelatin capsules with a balling gun), (2) Irradiated Dry Yeast (Type 142F, 142,000 units per gram, fed mixed in the grain concentrate mixture), and (3) Super Drex 3000 (irradiated ergosterol on a dry carrier, 6,614,000 units per gram, fed mixed in the grain concentrate mixture). All the concentrates were supplied gratis to the cooperating dairymen in capsules or packages containing the amount to be fed at individual feedings. Dosage level was not known by the dairymen.

Twenty-six purebred Jersey breeders were selected as cooperators, including the Jersey herd of the Ohio Agricultural Experiment Station. Only Jersey cows with at least one previous attack of milk fever were used.

According to the experimental plan the vitamin D concentrate provided was to be fed on a twice-a-day basis beginning five days before the expected calving date. If the cow calved before seven days of vitamin D feeding were completed, one feeding was to be given postpartum. If seven days of feeding were completed prior to parturition, vitamin D feeding was to be discontinued. Daily vitamin D feeding records were kept on data sheets provided for each cow. Additional information asked for on each data sheet included the date of birth, previous milk fever history, due date, date fresh, vitamin D concentrate used, key to the daily dosage, date, if milk fever occurred, and date of relapse, if any. The data sheets were returned to us and were used as the basis for analyzing the results. In all, complete data were obtained on 164 parturitions in the 26 herds.

RESULTS AND DISCUSSION

Examination of the data indicated that maximum protection could not be expected until vitamin D had been fed for about three days and that protection dropped off rapidly within a day after vitamin D feeding was stopped. On this basis, those cows that the records showed had been fed vitamin D for at least three days (six feedings), including the day of parturition, and those that had calved within one day after the last vitamin D was fed, were selected as the on-schedule group and used to calculate the protection obtained from the three vitamin D dosage levels. In the 164 parturitions reported there were a total of 113 parturitions in the on-schedule group. In the off-schedule group ten cows calved too early and 41 calved too late.

Table 1 shows the per cent incidence of, and per cent protection from, milk fever resulting from the feeding of the three vitamin D concentrates at the three dosage levels in the on-schedule group.

The calculation of per cent protection from milk fever was based on the control data obtained previously in the Ohio Agricultural Experiment Station

TABLE 1

Effect of different vitamin D dosages and three vitamin D concentrates on the incidence of and protection from milk fever in cows with previous milk fever histories that calved on schedule[a]

Vitamin D concentrate	No. calved normally	No. milk fever cases	% Milk fever incidence	% Milk fever protection[b]
	No vitamin D (controls)[c]			
None	14	25	64.1	00.0
	15 million units/day			
Viosterol (capsules)	32 (5.6)[d]	8 (5.3)	20.0	68.8
Irradiated yeast	13 (4.7)	4 (5.3)	23.5	63.2
Super Drex 3000	0	0	.....	.....
All concentrates	45 (5.3)	12 (5.3)	21.1	67.1
	20 million units/day			
Viosterol (capsules)	9 (4.4)	1 (4.5)	10.0	84.4
Irradiated yeast	0	0	.....	.....
Super Drex 3000	14 (6.0)	2 (7.0)	12.5	80.5
All concentrates	23 (5.4)	3 (6.4)	11.5	82.0
	30 million units/day			
Viosterol (capsules)	19 (5.8)	3 (6.7)	13.6	78.7
Irradiated yeast	2 (7.0)	1 (6.5)	33.3	47.9
Super Drex 3000	5 (5.6)	0	00.0	100.0
All concentrates	26 (5.9)	4 (6.7)	13.3	79.2

[a] On schedule refers to cows that were fed vitamin D for at least three days prepartal, including the day of parturition, and calved within one day after receiving the last vitamin D.

[b] Based on 64% milk fever expectancy in control Jerseys[c] with previous milk fever history.

[c] Data obtained from records of the Ohio Agricultural Experiment Station Jersey herd. Twenty-two parturitions during 1949-53 and 17 others prior to 1944, all with previous milk fever histories.

[d] Figures in parentheses indicate the average number of days vitamin D was fed prepartal including the day of parturition.

Jersey herd, Table 1. Data from two previous periods, one—17 parturitions prior to 1944 (unpublished data), and the other, 22 parturitions between 1949 and 1953 (3), showed per cent milk fever incidence in cows with previous milk fever history to be, respectively, 64.7 and 63.6. Of interest in this connection were the data obtained by Dell and Poulton (2), who found milk fever incidence to be 61.3% in 31 control cows with previous milk fever histories at the Maine Agricultural Experiment Station. Therefore, based on 64% milk fever expectancy in Jersey cows with previous milk fever histories, the per cent protection from milk fever was calculated as follows (Table 1):

Total No. of parturitions $\times$ 64% = No. milk fever cases expected.

No. of cases expected $-$ No. of cases observed = No. protected.

(No. protected $\div$ No. expected) $\times$ 100 = per cent milk fever protection.

Also indicated in Table 1, in parentheses, are the average numbers of days vitamin D was fed prepartal, including the day of parturition. The over-all average length of the dosage period was 5.6 days (11.2 feedings) for the on-schedule group.

In general, the numbers were too few to determine accurately any differences that might exist among the three vitamin D concentrates in relative

TABLE 2

Effect of duration of vitamin D feeding and dosage level on the incidence of and protection from milk fever

	Number of days vitamin D was fed													
	1		2		3		4		5		6		7[a]	
							15 million units/day							
Normal parturitions	0	.....	4	(35.0)[b]	6	(46.7)	9	(65.6)	13	(75.8)	5	(90.0)	12	(107.1)
Milk fever cases	1	(20.0)	0	.....	1	(50.0)	2	(65.0)	6	(75.8)	2	(95.0)	1	(120.0)
% Milk fever	100.0		00.0		14.3		18.1		31.6		28.6		7.7	
% Protection	00.0		100.0		77.7		65.3		50.7		55.4		88.0	
							20 million units/day							
Normal parturitions	0	.....	0	.....	4	(62.3)	5	(80.0)	2	(105.0)	6	(125.0)	6	(141.6)
Milk fever cases	0	.....	0	.....	0	.....	1	(90.0)	0	.....	0	.....	2	(140.0)
% Milk fever	..		..		00.0		16.7		00.0		00.0		25.0	
% Protection	..		..		100.0		74.0		100.0		100.0		60.9	
							30 million units/day							
Normal parturitions	0	.....	2	(60.0)	3	(90.0)	6	(127.5)	0	.....	5	(192.0)	12	(212.5)
Milk fever cases	..	.....	3	(70.0)	0	.....	0	.....	0	.....	2	(187.5)	2	(210.0)
% Milk fever	..		60.0		00.0		00.0		..		28.6		14.3	
% Protection	..		6.3		100.0		100.0		..		55.4		77.7	
							All dosage levels							
Normal parturitions	0	.....	6	(35.0–60.0)	13	(46.7–90.0)	20	(65.6–127.5)	15	(75.8–105.0)	16	(90.0–192.0)	30	(107.1–212.5)
Milk fever cases	1	(20.0)	3	(70.0)	1	(50.0)	3	(65.0–90.0)	6	(75.8)	4	(95.0–187.5)	5	(120.0–210.0)
% Milk fever	100.0		33.3		7.1		13.0		28.6		20.0		14.3	
% Protection	00.0		47.9		88.8		79.6		55.4		68.8		77.7	

[a] Includes cows that calved within one day after last vitamin D was fed, on-schedule group.

[b] Figures in parentheses indicate the average total units (millions) of vitamin D fed prepartal, including the day of parturition.

effectiveness. However, where it was possible to make direct comparisons of the protection afforded by the different concentrates, involving sufficient numbers (Table 1), there were no apparent differences. Therefore, the data obtained from all three concentrates were combined (Table 1) in calculating the average per cent incidence and average per cent protection for the three dosage levels.

TABLE 3

Effect of lapse of time between last vitamin D fed and parturition on the incidence of and protection from milk fever

	Day parturition occurred after last vitamin D fed[a]				
	1st[b]	2nd	3rd	4th-7th	8th-14th
			15 million units/day		
Normal parturitions	9	2	2	4	2
Milk fever cases	5	4	2	1	3
% Milk fever	35.7	66.7	50.0	20.0	60.0
% Protection	44.2	00.0	21.9	68.8	6.3
			20 million units/day		
Normal parturitions	8	2	1	2	0
Milk fever cases	0	2	1	3	1
% Milk fever	00.0	50.0	50.0	60.0	100.0
% Protection	100.0	21.9	21.9	6.3	00.0
			30 million units/day		
Normal parturitions	8	2	0	3	1
Milk fever cases	2	1	0	2	0
% Milk fever	20.0	33.3	..	40.0	00.0
% Protection	68.8	47.9	..	37.5	100.0
			All dosage levels		
Normal parturitions	25	6	3	9	3
Milk fever cases	7	7	3	6	4
% Milk fever	21.9	53.8	50.0	40.0	57.1
% Protection	65.8	15.9	21.9	37.6	10.7

[a] All calved after seven days of vitamin D feeding had been completed.

[b] Included with on-schedule cows (Table 1).

Approximately the same per cent protection was obtained at both the 20 and 30 million units per day dosage level, 82.0% and 79.2%, respectively, Table 1. However, at the 15 million unit per day dosage the average per cent protection was only 67.1%. Regression analysis of these data showed that there was no statistically significant difference in per cent incidence of or protection from milk fever among the three dosage levels. Because of the lower average per cent protection at the 15 million unit dosage level, indicating that 15 million units per day might, in future experiments involving larger numbers, prove to be less effective than either 20 or 30 million units per day, this level was considered too low to recommend for prevention on a practical basis. The 20 million unit per day dosage was as effective as 30 million units. Thus, it appears that the previously recommended dosage of 30 million units can be safely lowered to 20 million units per day without sacrificing protection, and future experiments may show the minimum effective dosage to be even lower.

That maximum protection from vitamin D feeding was not attained until at least three days of feeding had been completed is shown in Table 2. A statistically significant drop in protection resulted after one day had elapsed between last vitamin D fed and parturition, as shown in Table 3. As can be seen in Table 3, some protection was still apparent in the off-schedule cows that calved late, for several days after vitamin D feeding was terminated. These data substantiate our previous observation that little protection against milk fever can be expected before three days of feeding have been completed and that protection is rapidly lost following cessation of vitamin D feeding.

No adverse effects were reported that could be attributed to the feeding of the large doses of vitamin D. This indicates that sufficient margin of safety was afforded by limiting the vitamin D feeding period to seven days to avoid harmful effects that have been shown to result from longer periods of vitamin D feeding (1, 6).

Our data, Table 2, show that there was no statistically significant difference in per cent incidence of or per cent protection from milk fever between three and seven days feeding in the on-schedule cows. When the data in Table 2 were arranged according to total units of vitamin D fed prepartal, irrespective of vitamin D units fed per day, milk fever incidence did not increase at the higher prepartal dosages.

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EFFECT OF WHOLE-BODY γ-IRRADIATION ON THE CHARACTERISTICS AND METABOLISM OF BULL SEMEN DURING THE EARLY POST-IRRADIATION PERIOD[1]

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SUMMARY

Whole-body γ-radiation of mature Hereford bulls with a single dose of 400 r from a cobalt^{60} source did not result in any apparent change in semen characteristics (sperm concentration and initial fructose level) or in sperm activity (initial motility and fructolytic activity) during a 3-wk. post-irradiation period. Evidently, those cells which were past the radiosensitive spermatogonial stage at the time of irradiation were metabolically unaffected.

The effect of ionizing radiation on spermatogenesis has been extensively studied in laboratory animals by histological techniques (1, 2, 3, 8, 9, 13, 14, 19, 29). However, few data have been reported based on semen examinations, except for man, where some clinical reports are available (15, 17), and for the dog, where Casarett (4, 5, 6) has done extensive work on the effect of x-irradiation on semen characteristics and on actual fertility. Casarett found that whole-body x-irradiation of beagle dogs at 3.0 r per week for 40 to 60 wk. resulted in reduced sperm counts and sterility in mating tests, whereas doses of 0.3 r per week and 0.6 r per week over the same period had little or no effect. Murphree and Parish (12) reported that when mature beef bulls were exposed to a single dose of 100-400 r whole-body γ-irradiation, the first observed semen changes were an increase in per cent abnormal sperm at 6 wk. after irradiation and a decrease in sperm concentration at 8 wk. after irradiation.

These data are in accord with the concept that radiation selectively inhibits spermatogonial division. Therefore, it may be assumed that there would be no change in the characteristics of ejaculated semen until those cells which were beyond the sensitive stages of spermatogonial division at the time of irradiation have been ejaculated and those cells which were spermatogonia at the time of

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[2] The senior author was an Oak Ridge Institute of Nuclear Studies Research participant at the time this study was conducted.

irradiation, mature and become available for ejaculation. This leaves open the question of whether radiation has a direct effect on those cells which are past the point of spermatogonial division and particularly on those cells which have completed their development and are morphologically mature (i.e., testicular or epididymal sperm) at the time of irradiation. Irradiation would not affect the concentration or morphology of these mature sperm cells, even though it might have a direct ionizing effect on their protoplasm and, therefore, on their metabolic activity. Sperm motility and sperm metabolic activity are used as standard estimates of cell activity and viability, and the possibility that radiation affects testicular or epididymal sperm may be investigated by study of these semen parameters before and after irradiation.

MATERIALS AND METHODS

The semen specimens used in this study were collected by electro-ejaculation (16) from ten Hereford bulls, maintained on native pasture during the months of June and July, 1958, at the UT-AEC Agricultural Research Laboratory, Oak Ridge, Tennessee. Two consecutive specimens of approximately 5 ml. each were collected from each bull, three times a week, for a total of six specimens per bull per week.

The original plan was to study the semen during a preirradiation period, pair the bulls on the basis of semen characteristics, and irradiate one member of each pair. However, due to the wide variation among bulls exact pairing was not feasible. The preirradiation means of the parameters of semen quality of those bulls selected for irradiation were considerably higher than the means of those bulls that were not irradiated (Table 1). Nevertheless, it was felt that

TABLE 1

Effect of γ-irradiation on semen characteristics and on fructose utilization

Treatment periods	No. of bulls	No. of semen specimens	Initial motility	Sperm concentration per milliliter ($\times 10^6$)	Initial fructose level	Fructose utilized in		
						20 min.	40 min.	60 min.
			(%)		(mg/ml)			
Irradiated	5							
First period		90	57	0.306	3.27	0.28	0.49	0.62
Second period		94	54	0.285	2.03	0.24	0.40	0.51
Nonirradiated	5							
First period		72	49	0.200	2.45	0.23	0.37	0.47
Second period		100	55	0.204	2.03	0.26	0.42	0.55

these data would be of value if it was understood that direct treated-vs.-control comparisons would not be made but, rather, that Period One vs. Period Two comparisons would be made in both the irradiated and nonirradiated groups.

After a 3-wk. preirradiation period, during which semen was collected from all ten bulls, as described, five of the bulls were exposed to 400 r whole-body gamma irradiation, at the rate of 21 r per hour for 19 hr., from a cobalt^{60}

source designed for the irradiation of large animals (22). Semen was collected from all ten bulls for a 3-wk. post-irradiation period. The specimens were handled, diluted, incubated, and prepared for fructose assay as previously described (11). Fructose utilizations were determined after 20, 40, and 60 min. of incubation at 37° C., after the method of Roe (18), as modified (7). Statistical analyses of the data followed standard methods (21).

RESULTS

The means of semen characteristics and of fructose utilizations for both irradiated and nontreated groups during the pre- and post-irradiation periods are presented (Table 1). No effect of irradiation on semen specimen characteristics (sperm concentration or initial fructose level) or on sperm activity (initial motility or fructose utilization) is apparent. There is some suggestion (Table 1) that initial fructose level declined in the post-irradiation period, but this is also true for the nontreated group. An examination of the fructose level data by bull means indicates that although the mean decline in the irradiated group was 1.24 mg/ml (3.27-2.03), one bull showed an increase (+0.52 mg/ml), two bulls showed decreases (−0.74 and −0.53), and two bulls showed marked decreases (−1.71 and −3.63 mg/ml). It is suggested that this may have been due to the small number of animals involved (five irradiated bulls) and to the large order of variation in individual specimen and bull mean fructose levels before and after irradiation, since the single bull with the most marked decrease (−3.63 mg/ml) in fructose level accounts for the difference, −1.24 mg/ml for the irradiated group as opposed to −0.42 mg/ml for the nontreated group, in the pre- and post-irradiation changes in fructose level.

This is supported by an analysis of the effect of irradiation on initial motility, sperm concentration, and initial fructose level (Table 2). There was no significant effect of irradiation (T_I) on any of these three parameters, nor was there any apparent effect of environment (T_E). The significant ($P < 0.01$) BT_I term for fructose level (Table 2) indicates the large order of bull × treatment interaction mentioned in the discussion of Table 1.

TABLE 2

Analysis of variance of effect of γ-irradiation on semen characteristics

Source of variation	Symbol	Degrees of freedom	Mean square		
			Initial motility	Sperm concentration	Initial fructose level
Among bulls	B	9	4,784[c]	689,750[c]	496,468[c]
Environmental effect[a]	T_E	1	2,569	927	67,802
Irradiation effect[b]	T_I	1	498	19,887	530,834
B × T_E	BT_E	4	364	23,848	27,916
B × T_I	BT_I	4	474	122,240[d]	222,667[c]
Ejaculates in bulls	E	336	338	47,984	17,930
Total		355			

[a] Between treatment periods, using five untreated bulls.
[b] Between treatment periods, using five irradiated bulls.
[c] Statistically significant ($P < 0.01$).
[d] Statistically significant ($P < 0.05$).

An effect of radiation on fructose utilization might be mediated through radiation effects on motility, sperm concentration, or fructose level, since it has been shown (10) that all three of these semen parameters influence sperm fructolytic activity, or else it might be a direct effect on the metabolic activity of the cell not mediated through motility, sperm concentration, or fructose level. Since there was no apparent effect of radiation on the three semen parameters during the 3-wk. post-irradiation period, we may test directly for a radiation effect on fructose utilization. An analysis of variance (Table 3) indicates that there was no effect of radiation on fructose utilization.

TABLE 3

Analysis of variance of effect of γ-irradiation on fructose utilization

Source of variation	Symbol	Degrees of freedom	Mean square Fructose utilized in 20 min.	40 min.	60 min.
Among irradiated bulls	B	4	2,229[b]	7,242[b]	11,884[b]
Irradiation effect[a]	T_I	1	731	3,664	5,444
$B \times T_I$	BT_I	4	909[c]	2,561[c]	4,595[b]
Ejaculates in bulls	E	174	321	850	1,277
Total		183			

[a] Between treatment periods, using five irradiated bulls.
[b] Statistically significant ($P < 0.01$).
[c] Statistically significant ($P < 0.05$).

DISCUSSION

Whole-body radiation effects on ejaculated semen parameters (sperm concentration and motility) do not become apparent for 7 to 8 wk. post-irradiation in the bull (12) and for an even longer period in the dog (4, 5, 6). Once such effects become apparent and the sperm concentration and motility are depressed, the animal may become sterile for a variable period of time (depending on radiation dose) and then may recover in quality and fertility.

This study was designed to investigate the effects of radiation on semen characteristics (sperm concentration and initial fructose level) and also on sperm activity (initial motility and fructolytic activity), during the early post-irradiation period, in an effort to determine if those cells (epididymal and testicular sperm and, possibly, spermatids) which have already developed beyond the stage of extreme radiosensitivity are affected by ionizing radiation.

No effects of gamma radiation (400 r) on initial motility, sperm concentration, initial fructose level, or 20-, 40-, and 60-min. fructose utilizations were demonstrated in this study. Sperm collected from irradiated bulls during the early post-irradiation period show the same order of motility and metabolic activity as sperm collected prior to irradiation.

ACKNOWLEDGMENT

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EFFECTS OF ESTRADIOL BENZOATE INJECTIONS ON THE CHARACTERISTICS OF BOVINE SEMEN

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SUMMARY

Estradiol benzoate was injected into two normal bulls at levels ranging from 0.99 to 70 mg. per week. At levels up to and including 15 mg. per week, supplementary estrogen produced statistically insignificant increases in motility, per cent live spermatozoa, volume per ejaculate, spermatozoa concentration, and total spermatozoa per ejaculate. The per cent of abnormal spermatozoa was decreased but the decrease was not statistically significant. Levels of 35 and 70 mg. per week decreased semen quality. Seventy milligrams per week decreased the libido in one bull but had no effect on the libido of the other.

Gassner *et al.* (3) reported that small amounts of estrogen acted synergistically with testosterone in the production of fructose by the seminal vesicle in the castrate bovine, and that estrogen alone had no effect. The synergistic response was erratic, but seemed to depend on the interval between castration and treatment. Large doses (50 mg. of estradiol dipropionate at intervals of two to three days for 38 days) caused severe testicular degeneration and lowered the quality of bull semen. Those workers reported that a high percentage of abnormal spermatozoa appeared in the semen during treatment, but they gave no details on the types of abnormalities found.

Unpublished data from our laboratory indicate that bulls of subnormal fertility show differences in the types of abnormal sperm produced. Since estrogen is known to cause abnormalities in the sperm, experiments were designed to test the effects of estrogen on the semen from normal bulls.

EXPERIMENTAL PROCEDURE

Semen was collected with an artificial vagina from two normal mature Jersey bulls at weekly intervals. Two ejaculates were collected at each time. Semen collected before the injections began and after injections ceased was used for control data. Immediately after collection the volume of the ejaculate was determined, motility was estimated, a slide was prepared for vital staining, and aliquots were deproteinized for fructose and citric acid determinations. Live spermatozoa and abnormal spermatozoa were counted on slides stained according to the method of Mayer *et al.* (5). Concentration of sperm was determined by counting in a hemocytometer. Fructose was determined by the method of Mann (4), and citric acid by the method of Saffran and Denstedt (7).

Estradiol benzoate (courtesy of Schering Corporation) was injected intramuscularly into the bulls according to the following schedule: 0.99 mg. per week for 5 wk., 1.99 mg. per week for 3 wk., 4.99 mg. per week for 4 wk., 15 mg.

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per week for 9 wk., 35 mg. per week for 4 wk., and 70 mg. per week for 2 wk. Injections of 70 mg. were stopped after 2 wk. because one of the bulls became inactive and semen quality in both dropped severely. At levels from 0.99 to 15 mg. weekly the injections were given three times weekly; at 35 and 70 mg. weekly the injections were given daily. Semen was collected for 14 wk. after injections were stopped to determine the time required for the semen to become normal. These post-injection collections were divided into three intervals as follows: Interval 1 included 1 to 5 wk. following withdrawal; Interval 2 was from 6 to 10 wk.; and Interval 3 was from 10 to 14 wk.

RESULTS

The characteristics of the semen before, during, and following treatment with estrogen are shown in Figures 1 and 2. At levels up to and including

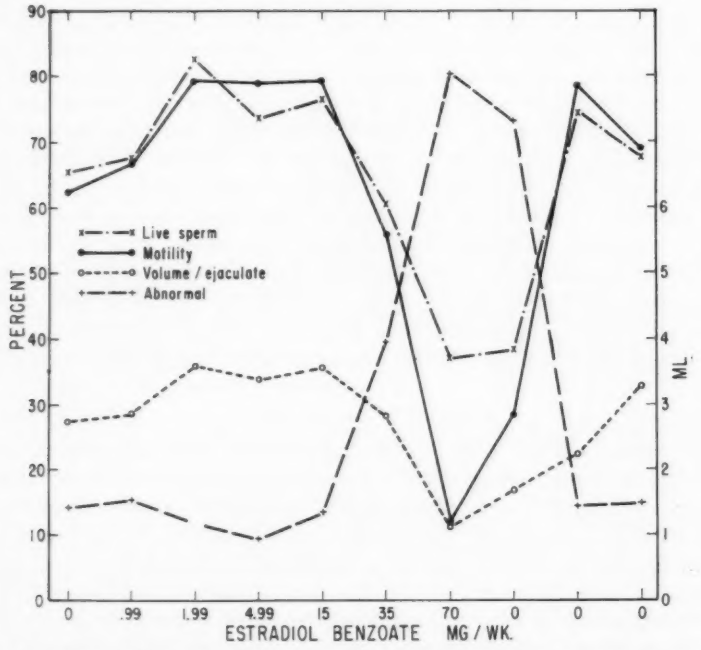

FIG. 1. Characteristics of semen from two normal bulls treated with estrogen. Abnormal spermatozoa, motility, and live spermatozoa, in per cent. Volume of ejaculate in milliliters.

15 mg. of estradiol benzoate per week, most of the characteristics of the semen improved slightly: There was a decrease in abnormal forms, and an increase in motility, volume per ejaculate, sperm concentration, and total sperm per ejaculate; fructose and citric acid concentrations did not change materially.

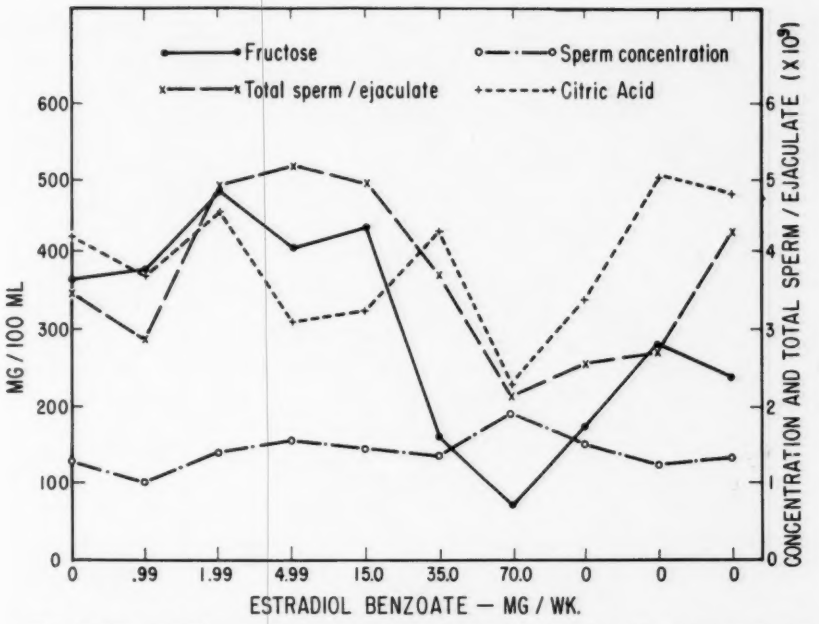

FIG. 2. Characteristics of semen from two normal bulls treated with estrogen. Concentration of spermatozoa is in billions per milliliter.

With a dose of 35 mg. per week the semen deteriorated. The effects were noted within 3 wk. of the beginning of injections, and the transition was sharply defined. For example, ejaculates collected the second week from one bull contained 23 and 21% abnormal spermatozoa and those collected the 3rd wk. contained 60 and 61% abnormals. A dose of 70 mg. per week decreased semen quality further.

The characteristics of the semen began to return to normal 5 wk. after the injections were stopped. By the 10th wk. the semen from both animals was essentially normal in most characteristics. Volume and total sperm per ejaculate seemed to return to normal values more slowly than the other characteristics measured.

The types of abnormal spermatozoa produced by estrogen are rather interesting. During the control periods one bull produced an average of 21.6% abnormal spermatozoa. Thirteen per cent were tailless, 3.5% had looped tails, 1% showed head irregularities other than pyriform, 2.6% had pyriform heads, and 1.5% had coiled tails. The other bull produced 8.4% abnormal sperm, with 2% tailless, 3% looped tails, 0.5% head irregularities other than pyriform, 0.8% pyriform heads, 0.5% with protoplasmic droplets, and 1.6% coiled tails. The percentage decrease in abnormals while the bulls were receiving low amounts of estrogens was caused by the decrease in the tailless group. Increases in the

FIG. 3. Abnormal spermatozoa from a bull treated with estrogen.

number of looped and bent tails were large at the higher levels of estrogen treatment (Figure 3). Bent tails increased from 0 to 10% and looped tails from 2 to 51% (average for both bulls). Many spermatozoa with bent tails also showed an enlargement at the distal end of the midpiece. A few spermatozoa without bent tails contained this distal enlargement. These enlargements were not seen in either of the bulls during the control periods. The number of tailless sperm increased from 7.5 to 17% during the last two periods of treatment with estrogen. The other types of abnormals did not change during the experiment.

DISCUSSION

Statistical analyses comparing each treatment period against the control period by Student's "t" method for each bull showed highly significant changes for all the semen characteristics studied at estrogen levels of 35 and 70 mg. per week. At the low levels of injection, the slight improvements in quality were not statistically significant.

Ortavant (6) found the length of the spermatogenic cycle in the bull to be 40-42 days and the time required to transverse the epididymis to be ten days. The time of the appearance of the changes in the sperm produced by the bulls under estrogen treatment indicates that the effects of estrogens at these levels were primarily on spermiogenesis. The lowered concentration of sperm and

the length of time required for return to normal sperm concentration indicate damage to other spermatogenic cells. The types of cells affected and the extent of damage could not be ascertained, however, because testicular sections were not taken.

The amount of estrogen required to damage the spermatozoa is larger than the amounts required to produce changes in cows. Adsell *et al.* (1) found that 0.125 mg. injected into castrate cows in three equal doses caused proestrous changes, and that 0.299 mg. injected for three days was the average amount required to produce estrus. Unpublished data from this station indicate that 1,000 rat units per day (1.1 mg. per week) suppress the activity of the ovaries in heifers. Since levels up to 15 mg. per week either had no effect or were slightly stimulatory, the relative sensitivity appears to be on the order of at least 15 times. Emmens and Parkes (2) found that androgens are antagonistic to estrogens, which may explain the large amounts of estrogen necessary to produce damage to the spermatozoa.

The data suggest that the effects of estrogen on the semen may be indirect, mediated through its effect on the production and release of gonadotropins by the pituitary. If the effects are due to changes in the production and release of gonadotropins by the pituitary, treatment with estrogen in combination with gonadotropins may afford a means of estimating the level of gonadotropin secretion in the bull.

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SURVIVAL RATES OF RAPIDLY FROZEN BOVINE SPERMATOZOA

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SUMMARY

Subsamples of semen from each of two ejaculates from each of 12 bulls were extended with a yolk-citrate-glycerol extender, equilibrated 5 to 6 hr. at 5° C., and placed directly in a freezing bath at temperatures of +5, 0, −5, −10, −15, −20, −25, −30, −35, and −40° C. The freezing bath was cooled at the rate of 0.8° C. per minute from +5 to −15° C., at a rate of 5° C. per minute from −15 to −40° C., and at the rate of 10° C. per minute from −40 to −75° C. The percentage of motile spermatozoa in ampules subsequently stored in a mechanical freezer at −85° C. for 60 days were, in order of decreasing temperature of the freezing bath at the time of transfer, 29, 21, 24, 27, 33, 36, 35, 35, 35, and 35. From these data it is concluded that time can be saved and a higher percentage of motile spermatozoa recovered by transferring the semen abruptly from +5° C. to a freezing bath ranging from −20 to −40° C.

Early work on the freezing of bull spermatozoa by Polge and coworkers (10, 11, 12) indicated that for maximum survival of sperm the rate of cooling between +5 and −15° C. should not exceed 2° C. per minute, but that the rate could be increased to 10° C. per minute from −15 to −79° C. Abrupt cooling from +5 to −79° C. resulted in poor survival. Other workers (3, 7, 8, 13) have obtained highest sperm survival by cooling at rates ranging from 1 to 4° C. per minute down to −79° C. This procedure is generally tedious, time-consuming, and not easily regulated manually.

Luyet and Keane (6) froze spermatozoa to −195° C. in a two-step procedure. When the terminus of the first step was −20° C. nearly all the spermatozoa died, as contrasted with 75% survival when the terminus of the first step was −27° C. Bialy and Smith (1) reported that a higher percentage of spermatozoa survived when they were frozen to −79° C. in a two-step procedure, with the first terminus at −20° C. than when they were frozen by the slow cooling procedure used as the control. A two-step procedure, with the first terminus at −15° C., was no better than the control. Recent studies by Larson and Graham (5) and Polge (9) indicate that the terminus of the first step should be lower when storage is to be at −195° C. than when it is to be at −79° C. The results suggested, that once the critical ranges were better established, a faster, simpler, and less harmful freezing procedure could be developed.

The studies reported here were undertaken to establish the optimum temperature of the first terminus in a two-step freezing procedure with subsequent storage at −85° C.

EXPERIMENTAL PROCEDURE

Design of experiment. Two ejaculates of semen were obtained from each of 12 bulls owned by the New York Artificial Breeders' Coop., Inc. Each of the 24 ejaculates was processed and frozen at ten different rates, and subsequently

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stored at about −85° C. in a mechanical freezer for periods of one, 30, and 60 days. At the end of each period two ampules per treatment were thawed and duplicate estimates of the percentage of motile spermatozoa were made from each ampule.

Processing and freezing of semen. The fresh semen was first partially extended (2) in a nonglycerol extender composed of a 2.32% sodium citrate dihydrate buffer, 20% egg yolk, 1,000 units of penicillin, and 1,000 μg. of dihydrostreptomycin per milliliter of extender. After glycerolating according to the procedure described by Bratton *et al.* (2), the final extender contained 7% glycerol by weight, and 14.2 million motile spermatozoa per milliliter. The glycerolated semen was equilibrated from 5 to 6 hr., during which time 1-ml. aliquots were dispensed by gravity into 1.5-ml. glass ampules, sealed and placed in wire baskets made of ⅛-in. mesh hardware cloth.

The first of the ten treatments, designated as the control, was taken from the cold room at 5° C. and placed in an alcohol bath adjusted to the same temperature. Then the bath was cooled according to the standard procedure of adding Dry Ice in amounts sufficient to lower the bath temperature 0.8° C. per minute from +5 to −15° C., 5° C. per minute from −15 to −40° C., and 10° C. per minute from −40 to −75° C. After the temperature of −75° C. was reached, the ampules were transferred to a mechanical freezer and stored at about −85° C.

The nine experimental cooling rates were achieved by placing ampules into the cooling bath when it reached 0, −5, −10, −15 −20, −25, −30, −35, and −40° C., respectively.

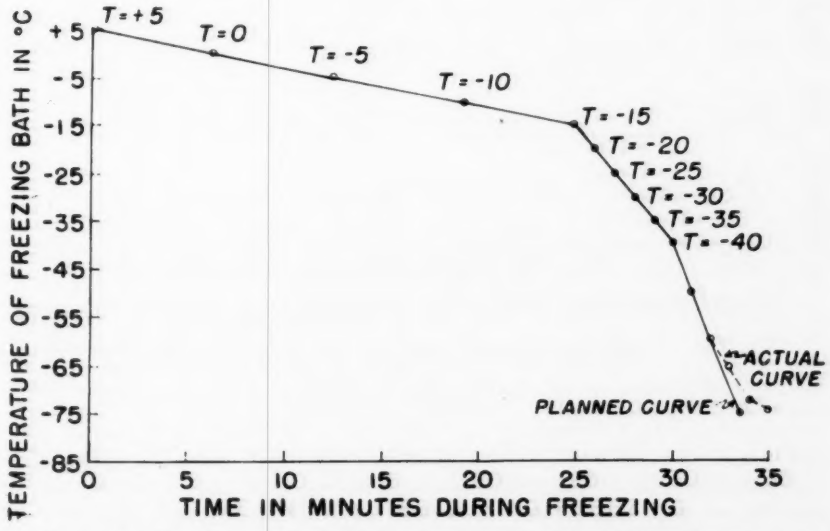

FIG. 1. Cooling and freezing rates and temperatures (T) at which ampules were transferred from +5° C. and placed in the freezing bath.

Examination of semen after storage. The duplicate estimations of the percentage of motile spermatozoa in each ampule were made within about 30 min. after thawing at 38° C. The identity of the samples was unknown to the persons estimating the percentage of motile spermatozoa.

RESULTS AND DISCUSSION

The average rate of cooling actually attained in this experiment and the standard curve which was being followed are shown in Figure 1. The numbers on the temperature curve indicate the temperatures of the freezing bath at the time each treatment was placed in the bath.

The average percentages of motile spermatozoa for the ten treatments after freezing and storage for various intervals of time are shown in Figure 2. Each

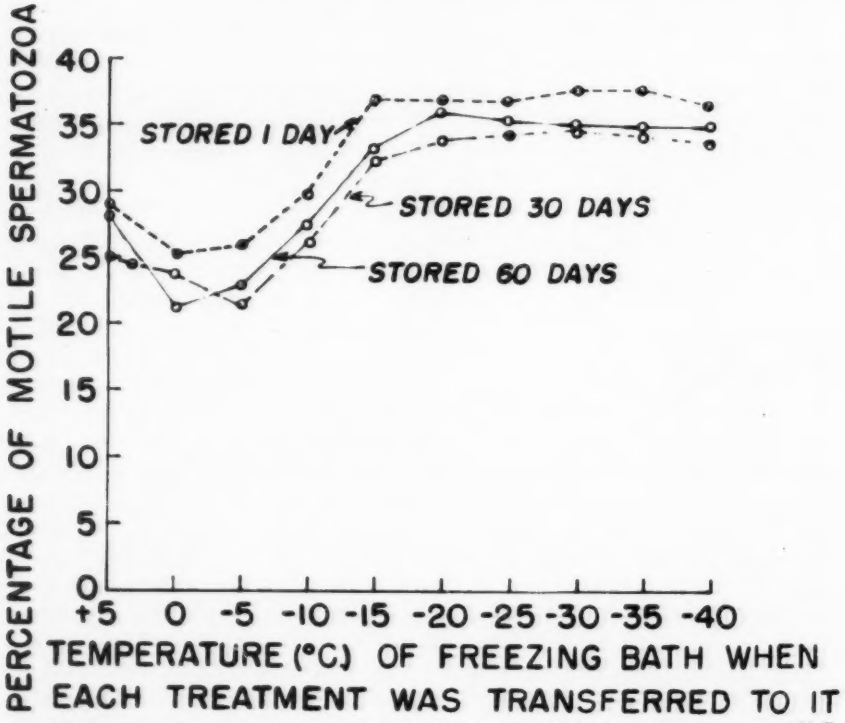

FIG. 2. Percentage of motile spermatozoa after 1, 30, and 60 days of storage at −85° C. when frozen at different rates.

point on the graph represents the average of 96 observations. The survival rate of samples placed in the freezing bath at temperatures between −15 and −40° C. was similar, and consistently higher than when the slower freezing procedures were used. This is in accord with the reports by Larson and Graham (5) and Polge (9).

The 13 sources of variation considered in the analysis of variance, and the corresponding expectations of the mean squares calculated according to Henderson's procedures (4), are shown in Table 1. The variances associated with rates

TABLE 1

Measured sources of variation and the expectations of their mean squares

Source of variation		Expectations of mean squares
Rates of freezing	R	$\sigma^2_w+\sigma^2_{m:aesbr}+k_1\sigma^2_{a:esbr}+k_5\sigma^2_{re:b}+k_8\sigma^2_{rb}+k_{12}\sigma^2_r$
Bulls	B	$\sigma^2_w+\sigma^2_{m:aesbr}+k_1\sigma^2_{a:esbr}+k_9\sigma^2_{e:b}+k_{11}\sigma^2_b$
Storage time	S	$\sigma^2_w+\sigma^2_{m:aesbr}+k_1\sigma^2_{a:esbr}+k_4\sigma^2_{se:b}+k_6\sigma^2_{bs}+k_{10}\sigma^2_s$
Ejaculates within bulls	E:B	$\sigma^2_w+\sigma^2_{m:aesbr}+k_1\sigma^2_{a:esbr}+k_9\sigma^2_{e:b}$
Interaction	R×B	$\sigma^2_w+\sigma^2_{m:aesbr}+k_1\sigma^2_{a:esbr}+k_5\sigma^2_{re:b}+k_8\sigma^2_{rb}$
Interaction	R×S	$\sigma^2_w+\sigma^2_{m:aesbr}+k_1\sigma^2_{a:esbr}+k_2\sigma^2_{rbs}+k_7\sigma^2_{rs}$
Interaction	B×S	$\sigma^2_w+\sigma^2_{m:aesbr}+k_1\sigma^2_{a:esbr}+k_4\sigma^2_{se:b}+k_6\sigma^2_{bs}$
Interaction	R×E:B	$\sigma^2_w+\sigma^2_{m:aesbr}+k_1\sigma^2_{a:esbr}+k_5\sigma^2_{re:b}$
Interaction	S×E:B	$\sigma^2_w+\sigma^2_{m:aesbr}+k_1\sigma^2_{a:esbr}+k_4\sigma^2_{se:b}$
Interaction	R×B×S	$\sigma^2_w+\sigma^2_{m:aesbr}+k_1\sigma^2_{a:esbr}+k_2\sigma^2_{rse:b}+k_3\sigma^2_{rbs}$
Interaction	R×S×E:B	$\sigma^2_w+\sigma^2_{m:aesbr}+k_1\sigma^2_{a:esbr}+k_2\sigma^2_{rse:b}$
Ampules	A:ESBR[a]	$\sigma^2_w+\sigma^2_{m:aesbr}+k_1\sigma^2_{a:esbr}$
Motility estimations	M:AESBR[b]	$\sigma^2_w+\sigma^2_{m:aesbr}$

[a] A = Ampules within ejaculates-bulls-storages-rates of freezing.
[b] M = Motility estimations within ampules-ejaculates-bulls-storages-rates of freezing.

of freezing and storage times were considered to be fixed and factorial, that for the bull source to be random, the ejaculate source random and nested within bulls, the ampule source random and nested within ejaculates-storages-bulls-rates, and the motility estimation source to be random and nested within ampules-ejaculates-storages-bulls-rates. These sources of variation are shown in the following mixed linear model.

$$X_{hijklm}=\mu+R_m+B_l+S_k+E{:}B_{jl}+RB_{lm}+RS_{km}+BS_{kl}+RE{:}B_{jlm}+SE{:}B_{jkl}+RBS_{klm}+RSE{:}B_{jklm}+A{:}ESBR_{ijklm}+M{:}AESBR_{hijklm}+E_{hijklm}$$

where X_{hijklm} is the value for the h^{th} motility estimation of the i^{th} ampule of the j^{th} ejaculate at the k^{th} storage time of the l^{th} bull and the m^{th} freezing rate. In this model μ is the mean of the population and E_{hijklm} are random errors with means of 0 and variances of σ^2_w. The other components of the model are assumed to be uncorrelated with mean of 0 and variance of σ^2_r, σ^2_b, σ^2_s, $\sigma^2_{e:b}$, σ^2_{rb}, σ^2_{rs}, σ^2_{bs}, $\sigma^2_{re:b}$, $\sigma^2_{se:b}$, σ^2_{rbs}, $\sigma^2_{rse:b}$, $\sigma^2_{a:esbr}$, and $\sigma^2_{m:aesbr}$.

The analysis of variance indicated that the differences among freezing rates, storage times, ejaculates within bulls, and ampules were highly significant ($P<0.01$). The difference between storage times reflects the difference between the one-day and subsequent storage intervals. The small nonsignificant rise between 30 and 60 days presumably resulted from sampling errors inherent in the method. Ampule differences were small, but were significantly different with 720/1440 degrees of freedom for performing the F-test. This suggests that small but real differences of unknown origin occur in the freezing of contents of ampules treated alike. Differences among bulls also were significant ($P<0.05$). The

TABLE 2

Estimates of the magnitude of the variance components for the percentage of motile spermatozoa

Components of variance	Computation of variances		Estimated variances
σ_r^2	$= R - R\times B/k_{12}$[a]	$= \frac{7,665-208}{288}$	$= 25.89$
σ_b^2	$= B - E{:}B/k_{11}$	$= \frac{7,102-1,953}{240}$	$= 21.45$
σ_s^2	$= S - B\times S/k_{10}$	$= \frac{2,845-369}{960}$	$= 2.58$
$\sigma_{e:b}^2$	$= E{:}B - A{:}ESBR/k_9$	$= \frac{1,953-59}{120}$	$= 15.78$
σ_{rb}^2	$= R\times B - R\times E{:}B/k_8$	$= \frac{208-178}{24}$	$= 1.25$
σ_{rs}^2	$= R\times S - R\times B\times S/k_7$	$= \frac{66-44}{96}$	$= 0.23$
σ_{bs}^2	$= B\times S - S\times E{:}B/k_6$	$= \frac{369-464}{80}$	$= -1.19 = 0$
$\sigma_{re:b}^2$	$= R\times E{:}B - A{:}ESBR/k_5$	$= \frac{178-59}{12}$	$= 9.92$
$\sigma_{se:b}^2$	$= S\times E{:}B - A{:}ESBR/k_4$	$= \frac{464-59}{40}$	$= 10.13$
σ_{rbs}^2	$= R\times B\times S - R\times S\times E{:}B/k_3$	$= \frac{44-89}{8}$	$= -5.62 = 0$
$\sigma_{rse:b}^2$	$= R\times S\times E{:}B - A{:}ESBR/k_2$	$= \frac{89-59}{4}$	$= 7.50$
$\sigma_{a:esbr}^2$	$= A{:}ESBR - M{:}AESBR/k_1$	$= \frac{59-43}{2}$	$= 8.00$
$\sigma_{m:aesbr}^2$	$= M{:}AESBR/k_0$	$= \frac{43}{1}$	$= 43.00$

[a] The constants k_0 to k_{12} represent the number of observations in the respective subclasses.

two-way interaction of rates × ejaculates within bulls and, the three-way interaction of rates × storage interval × ejaculates within bulls were highly significant ($P<0.01$).

The estimates obtained for the components of variance are shown in Table 2. These estimates show that motility estimations within ampules (m:aesbr), rates of freezing (r), bulls (b), and ejaculates within bulls (e:b), accounted for a major portion of the total variance. The large within ampule variance indicates that a minimum of two estimations of motility should be made in evaluating frozen semen. Since negative variances are not possible the two negative values that were obtained for b × s and r × b × s may be considered as estimates of parameters which are very close to or equal to zero.

The results of these studies show that the conventional slow procedures for freezing bovine spermatozoa may be speeded up by transferring the semen from +5° C. to a freezing bath ranging from −20 to −40° C., with a resultant saving in time and labor, and an increase in sperm survival at a storage temperature of −85° C.

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TECHNICAL NOTES

THE CONVERSION OF ALPHA-KETOISOVALERIC ACID TO VALINE BY LACTIC STREPTOCOCCI[1]

Streptococcus lactis, *Streptococcus cremoris*, and *Streptococcus lactis* var. *maltigenes* require valine for multiplication (1, 4, 7). The malty variant of *S. lactis* has been shown, in addition, to convert valine to 2-methylpropanal (6). If the degradation of valine follows a pattern similar to that of leucine by cells of *S. lactis* and its variant (4), then alpha-ketoisovaleric acid should fill the valine requirement of these organisms when substituted for the amino acid in a completely defined medium, and the formation of valine from its alpha-ketoacid analog should be demonstrable.

The methods followed to demonstrate the above precepts were similar to those described in an earlier publication (4).

The basal synthetic medium used was that of Niven (7), modified as follows: (a) the proportions of the amino acids found in casein (3) were substituted at levels comparable to those in casein hydrolyzate; and (b), sodium acetate was added to the medium as recommended by Collins *et al.* (2).

The nutritional studies involved three synthetic media: (a) complete basal; (b) basal minus valine; and (c) basal minus valine plus alpha-ketoisovaleric acid. In the preparation of the latter, mole for mole of the keto acid was added to replace its amino acid analog in the complete medium. The media were incubated at 32° C. for 48 hr. before inoculation to ensure sterility. The inoculation technique was that described earlier (4), and the growth responses of the various strains (one of *S. lactis*. A.T.C.C.7962, one of *S. lactis* var. *maltigenes* (M_1,) and one of *S. cremoris*, A.T.C.C.9625), after four transfers in the different media, were measured by the use of a Klett-Summerson spectrophotometer at 660 mμ, then converted to per cent light transmission. The growth responses are given in Table 1, and show that all test strains utilize the keto acid fully as well as the amino acid valine, and that valine was essential for the multiplication of the organisms.

Resting cells of the three cultures were incubated with buffered solutions (pH 8.0–8.3) containing alpha-ketoisovaleric acid, pyridoxal phosphate, and glutamic acid. These suspensions were incubated for 3 hr. at 32° C. After incubation, the cells were removed by centrifugation and the supernatants analyzed chromatographically for amino acids and acid carbonyl compounds (5).

Valine and alpha-ketoglutaric acid were shown to be present in the incubated cell mixtures containing glutamic acid and the alpha-ketoacid analog of valine, whereas no valine and little or no alpha-ketoglutaric acid were demonstrable in the mixtures of cells and the keto acid, cells alone, or cells and glutamic acid.

TABLE 1

Essentially of valine for *Streptococcus lactis*, *Streptococcus lactis* var. *maltigenes*, and *Streptococcus cremoris*, and the replacement of valine with alpha-ketoisovaleric acid

Cultures	Media		
	Basal	Basal minus valine	Basal minus valine plus alpha-ketoisovaleric acid
	[*Light transmission (%)*]		
S. lactis (A.T.C.C.7962)	56.8[a]	100	55.7
S. lactis var. *maltigenes* (M_1)	63.7	99	56.2
S. cremoris (A.T.C.C. 9625)	80.5	99.5	73.5

[a] Per cent light transmission.

From these findings, it was concluded that the cultures contain active valine–glutamic acid transaminase systems, and that alpha-ketoisovaleric acid can be a precursor of valine in the metabolism of lactic streptococci.

S. lactis var. *maltigenes* has been shown to degrade leucine and/or alpha-ketoisocaproic acid to 3-methylbutanal, (5) and valine to 2-methylpropanal (6). In this study, when cells of the malty variant were incubated with alpha-ketoisovaleric acid, 2-methylpropanal accumulated. The presence of this volatile carbonyl in the incubated mixtures was demonstrated by paper chromatography (6) and by the flash-exchange gas-chromatographic method of Ralls (8). In the latter technique, the crystallized 2,4-dinitrophenylhydrazones were heated with alpha-ketoglutaric acid in glass capillary tubes and the products volatilized directly into a gas chromatography unit.

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REMOVAL OF IODINE^{131} FROM MILK[1]

Milk and certain other foods have received considerable attention as a channel by which fission products enter the human body. Because of the ease of sampling in various parts of the country, and because it is produced throughout the year, milk is analyzed each month by the United States Public Health Service for the concentration of radioactive fission products. Though no I^{131} was reported in samples collected from 12 cities in October, 1959, figures in the fall of 1957, in two cities, were greater than 900 $\mu\mu$c/liter, or about one-third the level considered by the Public Health Service to be the maximum permissible concentration.

I^{131} could become a serious general problem in case of large amounts of world-wide fallout, or a serious local problem in case of a nuclear reactor accident. Fission produces a high I^{131} yield, which is strongly adsorbed and held by cellulose-like materials and by dusts, when in carrier-free form, and is concentrated in the thyroid gland (1). Dunning (3) has discussed the dose to the thyroid from radioiodine in fallout. Maximum permissible concentration for pasture grasses has been suggested (1, 12). Assuming no loss of I^{131} from the grass except by radioactive decay, the highest concentration in milk from cows grazing on such pasture is expected to reach its maximum value about three days following contamination (13).

In October, 1957, a nuclear reactor accident in England caused I^{131} to be deposited over an area of about 200 sq. mi. (4). The concentration of the nuclide in the milk from some sections of the area exceeded 0.1 μc. (100,000 $\mu\mu$c.) per liter and caused it to be restricted for human consumption for as long as 6 wk. Other food products from the area were monitored, but none condemned.

The maximum permissible concentration of 0.1 μc/liter of milk as used by the English authorities was based upon: (1) maximum exposure to the thyroid of 20 rads, (2) a thyroid weight of 1.5 g., (3) a 20% thyroid uptake of ingested I^{131}, (4) an effective half-life of eight days, and (5) milk consumption of one liter per day.

The condemnation of this milk, and the high I^{131} levels in milk in some sections of this country in 1957, emphasize the importance of developing means of utilizing a milk supply should the presence of radionuclides become a problem.

The utilization of contaminated milk would depend largely upon the nature of the contaminating fission products. If the only radionuclides present in prohibitive quantities were those with short half-lives, such as I^{131} (eight days), the milk might be manufactured into dairy products suitable for storing sufficiently long to allow the I^{131} to decay. Another possibility would be to remove the radionuclide from the milk.

Glascock (8) reported that none of the I^{131} administered to a lactating cow was combined with the fat or protein in milk but was in the iodide form, and Lengemann and Swanson (10) have reported that approximately 90% of the I^{131} in milk was in the inorganic form. English workers (2, 9) have removed I^{131} from milk by means of a strong base anion exchanger and the milk remained palatable. The present report is a further study of ion-exchange and vacuum treatment methods for the removal of I^{131} from milk.

EXPERIMENTAL PROCEDURE

Four Jersey cows were given single oral doses of 3.5 mc. each of I^{131} at 9 A.M. on the was chosen at a tracer level. The weight of first day of the experiment and milked at 8 A.M. and 4 P.M. daily. The amount of I^{131} given the cows was not meant to duplicate conditions as they may have existed near the Windscale atomic plant in October, 1957, but

[1] This manuscript is published with the permission of the Director of the University of Tennessee Agricultural Experiment Station, Knoxville, Tennessee. The radioactive materials used in this work were obtained from the Oak Ridge National Laboratory on allocation from the United States Atomic Energy Commission. The work was completed under Contract No. AT-40-1-GEN-242 between the University of Tennessee College of Agriculture and the Atomic Energy Commission.

radioactive iodine administered was approximately 0.0004% of the total iodine intake on the dosing day.

Individual liquid milk samples were analyzed for radioactivity by use of a scintillation counter. The percentage of the dose appearing in each milking was calculated for a seven-day collection period. The experiment was first conducted in July using two cows, and subsequently repeated in September using two other animals.

The milk was mixed and separated at 35° C. by use of a De Laval Junior Model separator and the radioactivities of the cream and skimmilk determined.

The mixed whole milk was exposed at room temperature to Dowex I-X8, 20-50 mesh in the chloride form by agitating 100 ml. of the milk and 5 g. of resin in a 150-ml. beaker for 10 min. The milk was filtered to remove the resin, and then analyzed for radioactivity. Fresh resin in the chloride form was used for each trial.

The effect of this treatment on the chloride and phosphorus content of the milk was determined in a duplicate experiment conducted in the same manner. The chloride contents of the control and treated milks were determined by titration with 0.05 *N* $AgNO_3$, using an 18-g. sample and 2 ml. of 10% potassium chromate as an indicator. The phosphorus contents were determined by the method of Fiske and Subbarow (6), using ashed samples.

To determine the volatility of I^{131} as it exists in milk, mixed milk was heated to 70° C. and allowed to impinge against the inner surface of a vacuum flask maintained at a vacuum level of 23 to 25 in. of mercury.

RESULTS AND DISCUSSION

The concentration of I^{131} in the cream was considerably below that in the milk before separation. The ratio of the concentration per milliliter of cream over the concentration per milliliter of milk for the July experiment was 0.60 ± 0.05, and for the September experiment was 0.65 ± 0.04. The concentration in the skimmilk was slightly higher than that in the whole milk. The concentration in the skimmilk, divided by the concentration in the whole milk in the July experiment, was 1.06 ± 0.02 and in the September experiment was 1.05 ± 0.01.

A large part of the I^{131} was removed by treatment with Dowex I in the chloride form. Irrespective of the level of I^{131} in the milk, the ion exchange method used in these experiments resulted in the removal of $89.8 \pm 2.5\%$ of the I^{131} in the July experiment and $93.0 \pm 1.5\%$ in the September experiment.

The average chloride content of the nine samples of milk was 124.4 ± 7.6 mg. per 100 g. milk, which after anion exchange treatment was 188.4 ± 4.2, an increase of 64.0 ± 4.7. The average phosphorus concentration was 100.9 mg. per 100 g. milk on the original milk, and 88.6 ± 7.3 on the treated milk, a decrease of 12.3 ± 3.9.

Figure 1 shows that milks having low chloride contents gained more chloride due to anion exchange treatment than did milk containing higher concentrations of chloride. Figure 2 shows that milk which gained the most chloride lost a smaller amount of phosphorus than other samples. This might be partially explained on the basis of a high phosphorus content in those samples having a high chloride content ($r = 0.46$). The average gain in chloride was 1.81 ± 0.13, and the loss of phosphorus was 1.98 ± 0.63 meq/100 g. milk.

No other data were collected in this study on other changes which might have occurred in the composition of the milk due to the ion exchange process. The vacuum treatment did not remove I^{131} from the milk.

The per cent of the dose appearing in the milk for the seven-day period was 15.2 and 15.6 for the two cows in the July experiment, and 4.8 and 8.6 in the September experiment. The quantity of milk secreted was 87.7, 42.4, 55.1, and 71.1 liters, respectively. This shows considerable variation in total secretion of I^{131}, which can not be entirely attributed to the quantity of milk secreted. Six per cent of a single oral dose has been reported to have been secreted in the milk in a seven-day period (10), and 12.5% has also been reported to have been secreted in the first 72 hr., the higher secretion rate being explained on the basis of a larger milk production (16). Glascock (8) reported 4.77% of a single dose was secreted in the milk in 15 days and Steenberg (15) reported 2.73% secreted in four and one-half days (nine milkings). Squire *et al.* (14) gave nine cows single

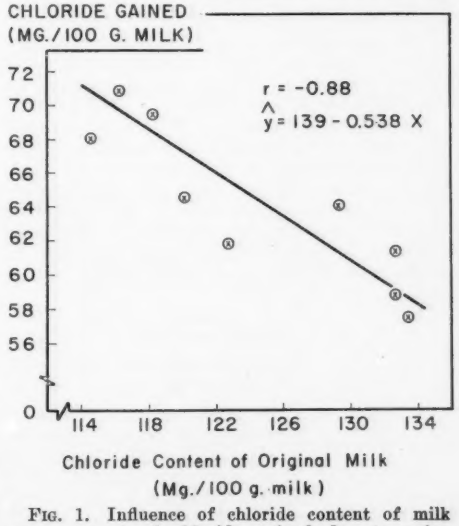

FIG. 1. Influence of chloride content of milk upon amount of chloride gained due to anion exchange treatment.

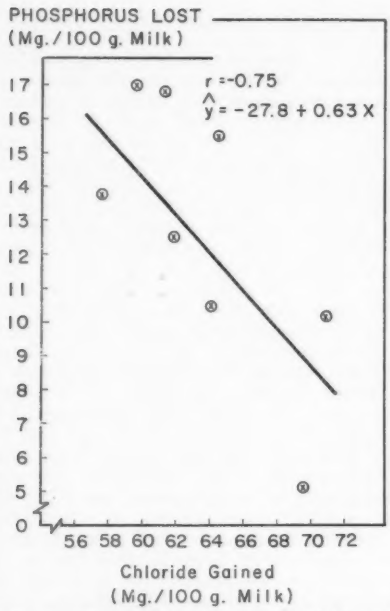

FIG. 2. Relationship between chloride gained and phosphorus lost due to anion exchange treatment of milk.

oral doses of I^{131} and recovered from 1.25 to 13.1% of the dose in the milk of the different cows in six days. Twenty cows given single oral doses by Garner and Sanson (7) secreted from 1.3 to 13.1% (mean of 5%), of the dose in the milk in six days. Seasonal variations also have been reported (11).

The concentration of I^{131} in the milk decreased with succeeding milkings at a rate closely resembling a logarithmic pattern, as was found by earlier workers (8, 15).

These data indicate that I^{131} can be removed from milk in a manner similar to that reported for strontium removal (5), that vacuum treatment of milk will not lower the concentration of I^{131}, and that the amount of I^{131} secreted in the milk is quite variable, ranging in this study from 4.8 to 15.6% of the single oral dose in seven days. The reasons for this are not apparent.

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INTERPRETIVE SUMMARIES OF PAPERS

QUANTITATIVE DETERMINATION OF CARBOHYDRATES IN ICE CREAM BY PAPER CHROMATOGRAPHY

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The analysis of carbohydrates in ice cream has presented considerable difficulties because of the complex nature of sugar combinations frequently used in the formulation of frozen desserts. Available analytical techniques do not distinguish between the different types of sugars and it was, therefore, necessary to develop a procedure by which individual sugars could be isolated from the mixture and quantitatively analyzed.

Separation was accomplished by descending paper chromatography of an ice cream sample which only required dilution with water. The following sugars could be separated on a single strip of chromatographic paper: fructose, glucose, galactose, sucrose, maltose, lactose, and higher sugars. The individual sugars were eluted from the paper with water and a quantitative determination was performed photometrically using the anthrone reaction. Standard curves were prepared for all sugars except maltose and the higher sugars which were analyzed by means of the standard curve for glucose.

Corn syrups contain higher sugars which, at the level of usage in ice cream, are present in quantities too small for analysis of each component. It is, however, feasible to elute the entire group of higher sugars and analyze them as though they were a single component. By this means, and by observing the amounts of glucose and maltose present, it is possible to deduce if a corn syrup was present and its type.

The determination is not simple, but it is no more involved than other techniques employing chromatographic separation with subsequent elution and analysis.

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A SIMPLIFIED METHOD FOR THE DETERMINATION OF IODINE^{131} IN MILK

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Radioiodine is one of the fission products formed as a result of nuclear detonations or reactor operations and may reach man through a variety of ecological systems. Iodine ^{131} (I^{131}) is considered as one of the hazardous isotopes in spite of its short half-life of about eight days.

Since I^{131} is metabolized by the cow and occurs in milk, analysis for this radionuclide in routine radiation monitoring programs is desirable. The available chemical and gamma ray spectrometric methods were found to be time-consuming or to require expensive instrumentation. Therefore, a nonspectrometric method which may be used in conjunction with nonashing method for strontium^{90} analysis, described elsewhere, was developed.

Most of the I^{131} in milk is in the inorganic form; however, the addition of a small amount of formaldehyde causes 98% $\pm$ 2% of the I^{131} to become bound so firmly to the milk proteins that it is not removed by any of the protein-precipitating reagents, as are the protein-bound alkaline earths. Accordingly, radioactivity associated with the proteins is radioiodine and may be determined by any gamma-detecting instrument, such as a shielded NaI crystal, appropriately coupled to an ordinary decade scaler.

The following method of preparing milk samples for I^{131} analysis has been devised.

(a) Add 1.0 ml. of 40% formaldehyde solution to 1 liter of milk and hold at room temperature for 2 hr.
(b) Treat milk with an equal volume of 24% trichloroacetic acid.
(c) Remove fat and proteins by filtration, and wash them with dilute trichloroacetic acid, (d) transfer quantitatively to a suitable polyethylene container, and (e) determine I^{131} activity by gross γ-counting.

A POTENTIOMETRIC METHOD FOR THE DETERMINATION OF CHLORIDE IN MILK

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The chloride content of milk is an index of normality. Colostrum, mastitis milk, and milk from cows in the last stages of lactation are abnormally high in chloride. The chloride content is also an index of flavor, because high chloride values are associated with low milk sugar. Milk abnormally high in chlorides may taste salty and be lacking in sweetness.

At present, chlorides are usually determined by titration with silver nitrate, using potassium chromate as an indicator. The method is fast, but it has two major disadvantages. The color change at the end of the titration is not very distinct. Different analysts may titrate to different degrees of color and obtain results which are consistently different. Furthermore, this method gives results which may be 30-40% too high because of interference by the protein.

In this paper, it is shown that proteins do not interfere appreciably if the titration is carried out in a nitric acid solution at pH 2, or if it is performed in the presence of a large excess of alum. The ordinary chromate indicator can not be used under these conditions, but a silver electrode attached to a pH meter gives very sharp end points which can be matched accurately by different analysts. The method is recommended for its accuracy and simplicity.

A STUDY OF THE RIPENING OF DARIWORLD AND CHEDDAR CHEESE WITH SPECIAL EMPHASIS ON PROTEOLYSIS

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Smooth, waxy consistency and desirable properties for processing develop more rapidly in Dariworld than in Cheddar Cheese. It is commonly known that Cheddar Cheese which lacks proper acid in manufacturing develops satisfactory consistency very slowly. This study was made to determine if the changes in the protein of these two varieties of cheese might explain the differences between these varieties and between normal cheese and that made with less-than-normal acidity.

Measurements of soluble nitrogen by the Kjeldahl method were made during 150 days of curing. Formol titrations were also made of acidified and filtered sodium citrate extracts of the cheese; these measurements indicate the progress of protein decomposition into the simpler compounds associated with extensive ripening. These tests, as well as measurements of acidity and scorings of the cheese, were made after 10, 30, 60, 90, and 150 days of curing.

The results obtained were unexpected because the normal Cheddar and Dariworld and the sweet Cheddar Cheese were practically alike by both measurements of protein changes. The sweet Dariworld showed more extensive protein changes than the three other modifications. Both of the low-acid lots were hard and woody in consistency and did not improve during ripening.

It was concluded that the rapid loss of curdiness and the early appearance of smooth consistency in normal Dariworld cheese can not be attributed to more rapid changes in the cheese protein. Normal acidity development is essential in both Dariworld and Cheddar, to obtain desirable body characteristics. Finally, it seems probable that the prolonged exposure of the Dariworld curd to the salt brine and the high moisture content of this cheese account for the rapid development of its characteristic consistency.

EFFECTS OF AGE AND DIET ON THE SECRETION OF PREGASTRIC ESTERASE IN CALVES

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The hydrolysis of dietary fat during the digestive processes is presumed to be a prerequisite for fat absorption. This chemical action is effected by lipolytic enzymes from various sources, particularly the pancreas, and to a lesser extent, the stomach and the small intestine. In the calf, such an enzyme, which is called pregastric esterase, is found in the salivary secretions also. Nothing is known, however, about the relative importance of pregastric esterase in fat

digestion. Moreover, little information is available on the factors that affect its rate of secretion. Therefore, the effects of the following factors on the secretion of pregastric esterase were investigated: (1) age of the calf (15 to 180 days), and (2) diet on which that calf is sustained (whole milk versus hay-grain). Six calves, three on each diet, were employed in this study. Enzyme secretion was measured while the calf was nursing, regardless of diet. Within the limits of this experiment, neither age nor sustenance diet had any noticeable effect on the output of pregastric esterase. Limited observations at 1 yr. of age suggest that there is still no change from the initial level in secretion. The output of pregastric esterase during the actual consumption of hay and grain, which is based on a few observations only, appeared to be considerably less than that stimulated by the nursing process. The results of this study, though they do not provide direct evidence on the importance of pregastric esterase, suggest that this enzyme may hydrolyze more than just trace amounts of dietary fat in the calf.

EFFECTS OF CONTINUOUS NURSING, LENGTH OF THE NURSING PERIOD, AND RATE OF MILK CONSUMPTION ON THE SECRETION OF PREGASTRIC ESTERASE BY CALVES

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Inasmuch as pregastric esterase, a lipolytic enzyme found in the salivary secretions of calves, may be involved in fat digestion to an appreciable extent, the study of factors affecting its secretion was continued. When 50 lb. of milk was fed continuously to calves, as ten successive 5-lb. increments, the esterase content of each increment decreased progressively. This decrease in secretion rate suggests that pregastric esterase accumulates in the secretory tissue during the interval between feedings. The quantity of enzyme secreted into a single 5-lb. unit of milk, however, was increased markedly by increasing the length of the nursing period. For example, when the 5 lb. were consumed in less than a minute, the secretion of pregastric esterase was only one-third of that when the nursing time was extended to nearly 11 min. The rate of milk consumption per se had but little effect on secretion, although a slight increase in output was noted when consumption rate was accelerated. The secretion of pregastric esterase was higher at the morning feeding (8 A.M.) than at the afternoon feeding (4 P.M.). The cause of this difference is unknown, although it may be related to the difference in length of interval between feedings—8 versus 16 hr. The foregoing observation on the effect of nursing time on pregastric esterase secretion poses the question of whether a calf digests milk fat more effectively when the milk is consumed slowly than when it is consumed rapidly. This question has no answer in present observations, but it does indicate the need for further research in this area.

RELATIVE GROWTH AND APPEARANCE OF YOUNG DAIRY CALVES FED TWO LEVELS OF MILK WITH A SIMPLE OR COMPLEX CALF STARTER

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Since 1900, replacement heifers on dairy farms have been fed less and less milk and have been encouraged to eat more dry calf starter and hay in an effort to save on feed costs.

The present experiment was designed to measure the rate of gain and to observe the appearance of Holstein heifers fed a low level of milk (250 lb.) while being fed a simple concentrate mixture of corn, oats, soybean oil meal, and wheat bran with added minerals. This low-milk, simple starter system was compared to the widely accepted 350 lb. of milk, complex calf starter system. To evaluate the diets over as broad a set of conditions as possible, calves were fed during two seasons of 1957, and were kept in three widely scattered calf barns. One barn was divided into pens and tie stalls, whereas the other two had only pens.

The appearance of the calves was rated as acceptable or unacceptable.

The calves on the low-milk scheme gained at a slightly slower rate than those fed 350 lb. of milk, but most were over the minimum rate of gain associated with thrifty appearance, i.e., 1 lb. per day. About 16% of the calves fed the low-milk level were slow in beginning to eat calf starter, and would have required additional care in order to do as well as the rest. For this reason, the low level of milk feeding is recommended only to the more skillful calf feeders.

The simple starter was equally as good as the complex starter at both levels of milk, in consumption, calf growth, and total digestible nutrient content.

The 250-lb. milk, simple starter system cost 28% less than the 350-lb. milk, complex starter system, primarily due to the decreased amount of milk fed.

EFFECTS OF FEEDING AUROFAC[1] UPON THE GROWTH OF SINDHI × JERSEY MALE CALVES TO SIX MONTHS OF AGE

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The influence of Aureomycin on the growth and well-being of young dairy calves in the United States has been well established. Most of the work has

[1] Trade name for product containing 1.8 g. Aureomycin and 1.8 mg. vitamin B_{12} per pound.

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been with calves under 16 wk. of age. With older calves there is much more variation in results than with younger calves. The slow rate of growth of most calves in India greatly increases the age of calving of heifers.

The purpose of this study was to determine if feeding Aurofac would speed up growth rate of calves when fed to 6 mo. of age. Aurofac was fed in milk at the rate of 5 g. per day from four days to 4 wk. of age, and added to the grain mixture at the rate of 20 lb. per ton from 4 wk. to 6 mo. After the 4th wk., the treated calves gained at a significantly higher rate than the untreated calves to 24 wk. The difference in growth rate increased steadily, with the greatest difference being at 24 wk. The calves fed Aurofac gained 18% more in weight and 23.6% more in height at withers than untreated calves during the 24-wk. feeding period.

SOME FACTORS AFFECTING AGE AT PUBERTY AND THE FIRST 90 DAYS OF LACTATION IN HOLSTEIN HEIFERS

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In this study of Holstein heifers, earlier age at puberty was associated with heavier 6-mo. weight and to a lesser extent with increased weight gain between 6 and 12 mo. of age. Genetic effects of sire line and system of mating on age at puberty were found; however, the statistical significance of these effects was removed by adjusting individual puberal ages for 6-mo. weight. This indicates that the effects of sire line and system of mating are influencing age at puberty through the 6-mo. weight. The occurrence of calfhood scouring reduced the 6-mo. weight in all heifers but delayed puberty in only the fall-born animals. In the group of nonscouring outbred heifers from outbred dams, the animals born in the spring attained puberty the earliest.

Heavier calving weight was associated with increased 90-day milk production. There was also an indication of increased 90-day milk production being associated with increased 6-mo. weight. Earlier age at puberty and heavier calving weight were associated with an increase in the average 90-day butterfat percentage.

From these results it appears that growth, attainment of puberty, and production early in the first lactation are interrelated. Heifers having heavier 6-mo. weights and calving weights and earlier ages at puberty averaged more milk and a higher butterfat percentage in the first 90 days of their lactation than the animals weighing less and reaching puberty later.

EFFECT OF CHLORPROMAZINE ON THE PERFORMANCE OF LACTATING DAIRY COWS

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Tranquilizing agents have become widely used in veterinary practice during the last few years and some of them have shown promise of becoming useful feed adjuncts. Since the amount of work conducted on the use of tranquilizers for dairy cows is limited, and no work had been reported on the effect of orally administered chlorpromazine on dairy cows, the present study was undertaken to obtain information concerning the performance of lactating cows fed this tranquilizer.

In the first phase of the study, graded levels of the tranquilizer were fed to six cows during different periods for the purpose of determining the dosage necessary to produce tranquilization in the mature dairy cow. Feeding as much as 5 g. of chlorpromazine per cow per day in two equal portions, at the morning and evening feedings, failed to produce tranquilization in Holstein cows. On the other hand, two cows fed single doses of 4.5 and 9.0 g., respectively, of the tranquilizer, showed a marked degree of tranquility. No chlorpromazine or its major metabolite, chlorpromazine sulfoxide, was detected in the milk of the cows at any level of administration. Thus, no danger of residues in the milk due to using this tranquilizer is visualized.

To determine the effect on milk production of feeding a low level of chlorpromazine over an extended period, 24 additional cows were fed 0, 250, or 500 mg. per cow per day of the tranquilizer, over a 24-wk. period. No appreciable differences were observed among the respective treatment groups with regard to milk production or body weight changes during the 24-wk. experiment. The total production of 4% fat-corrected milk for the three groups was 4,524, 4,752, and 4,244 lb. per cow, respectively. Thus, no advantage in milk production attributable to the feeding of the tranquilizer, chlorpromazine, was evident in this study.

STUDIES ON MILK FEVER IN DAIRY COWS. VI. EFFECT OF THREE PREPARTAL DOSAGE LEVELS OF VITAMIN D ON MILK FEVER INCIDENCE

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A field study involving 164 parturitions in Jersey cows, all with previous milk fever histories, in 26 Ohio herds was conducted to obtain further information on how vitamin D must be fed prepartal to provide maximum protection against milk fever. The assigned vitamin D dosage, 15, 20, or 30 million units/

day, was fed on a twice-a-day basis beginning approximately five days before the expected calving date and for one feed after parturition. If seven days of feeding were completed before parturition, vitamin D feeding was terminated. On-schedule cows were those that received at least three days of vitamin D feeding and calved within one day after termination of vitamin D feeding. In all, there were 113 in the on-schedule group. In the off-schedule group ten cows calved too early and 41 calved too late. Three vitamin D concentrates were used: Viosterol (capsules), Type 142F Irradiated Dry Yeast, and Super Drex 3000.

The average per cent protection for the three dosages based on 64% milk fever expectancy in Jersey cows with previous milk fever histories was 67.1% for the 15 million unit level, 82.0% at the 20 million unit level, and 79.2% at the 30 million unit level. No statistically significant differences were found in milk fever incidence or per cent protection among the three vitamin D dosage levels. Vitamin D was fed for an average of 5.6 days (11.2 feedings) prepartal, including the day of parturition in the on-schedule group. Because the 15 million unit/day dosage level resulted in a lower average protection than the two other dosages it was considered unwise to recommend less than 20 million units per day, until more numbers can be studied. There was no evidence, however, that 30 million units per day would be more effective than 20 million units per day. All three vitamin D concentrates used appeared to be equally effective when consumed. Certain cows did not accept irradiated yeast readily. The viosterol capsules had the advantages gained from forced feeding, and Super Drex 3000 presented no palatability problem.

Evidence obtained indicated that protection increased for the first three days of vitamin D feeding, remained at a high level after the third through the seventh day, and dropped precipitously ofter one day had elapsed following cessation of vitamin D feeding.

EFFECT OF WHOLE-BODY γ-IRRADIATION ON THE CHARACTERISTICS AND METABOLISM OF BULL SEMEN DURING THE EARLY POST-IRRADIATION PERIOD

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An increase in interest in the effects of radiation has followed the increase in the production and use of radioactive materials. Although all the tissues of the body are affected by radiation, those tissues in which cells are going through rapid cycles of division and proliferation are most susceptible to radiation damage.

The normal testis produces large quantities of sperm and is full of tubules containing many rapidly dividing cells. It has been shown that radiation selectively blocks sperm development at an early stage called spermatogonial division and, as a result, there is no replacement of sperm and a consequent drop in semen quality and fertility.

The effect of radiation on those cells which are past the radiosensitive spermatogonial division stage, i.e., the spermatids and sperm, has not been determined. It was the purpose of this study to examine bull semen before and after irradiation of the bulls, to determine if the radiation affected sperm cell metabolism or motility, two measures of cell activity.

Ten Hereford bulls were used and six semen specimens per week were collected by electroejaculation for a control period of 3 wk. to determine the normal production of semen by these bulls. Then five of the bulls were exposed to 400 roentgens of whole-body gamma irradiation from a cobalt-60 source. Six semen specimens per week were then collected by electroejaculation for a 3-wk. post-irradiation period.

There was no measurable effect of this dose of radiation on sperm activity (motility and metabolic activity) during a 3-wk. post-irradiation period. Evidently, those cells which were past the radiosensitive spermatogonial stage at the time of irradiation were metabolically unaffected.

EFFECTS OF ESTRADIOL BENZOATE INJECTIONS ON THE CHARACTERISTICS OF BOVINE SEMEN

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The histological condition of the testes and the different types of abnormal spermatozoa in the semen of bulls with impaired fertility indicate that different factors may be causing the low efficiency in the different animals. For example, the most frequent abnormality in the spermatozoa from bulls with infections of the genital tract is tailless spermatozoa. The semen from bulls with an inherited type of testicular hypoplasia contained large numbers of spermatozoa with coiled tails and head and midpiece abnormalities in addition to tailless spermatozoa. In other bulls with spontaneous breeding failure the most frequent type of abnormality is looped tails.

The physiological mechanisms responsible for these changes in the testes and semen from bulls are not known at the present time. Some investigators believe that the differences found with respect to the types of abnormal spermatozoa in the semen are quantitative in nature and represent the extent of damage to the seminiferous epithelium. Others believe that these differences may represent qualitative changes in the physiological processes occurring during spermatogenesis. Because some of the changes found in the semen from the low-fertility animals may be caused by endocrine imbalances, a series of experiments is in progress to determine the effects of steroid hormones on the charac-

teristics of the semen from normal bulls. The present paper reports the effects of estrogen on the semen from two normal bulls.

The bulls were injected with estrogen at levels ranging from 0.99 mg. per week to 70 mg. per week. The effects of supplementary estrogen differed at different dose levels. At low levels, estrogen caused a slight improvement of the quality of the semen as determined by the characteristics measured. However, these changes were not significant statistically. At higher levels, estrogen caused a marked decrease in the quality of the semen produced and characteristic abnormalities were found in the spermatozoa. The results are consistent with the theory that estrogen at low levels increases the synthesis and release of gonadotropins from the pituitary and that high levels are inhibitory. The experimental design was not adequate to determine if estrogen has effects not related to pituitary activity. If the effects of estrogen are mediated through its effect on gonadotropin secretion, it could be used to determine secretion rates in conjunction with injections of gonadotropin. The amount of estrogen required to interfere with spermatogenesis appears to be larger than the amount necessary to interfere with ovarian activity. This sex difference has been attributed to the androgen excretion in the male, but this theory has not been tested experimentally in the bull.

SURVIVAL RATES OF RAPIDLY FROZEN BOVINE SPERMATOZOA

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The development of frozen semen has made it possible to preserve the fertility of ejaculated bull semen for long periods of time. This tends to increase the number of progeny possible per sire, and when applied to outstanding sires may increase the rate of genetic progress. Partially negating this advantage has been the mortality of spermatozoa in the freezing process, making it necessary to initially include more viable spermatozoa per breeding unit for frozen semen than for unfrozen semen, thereby reducing the breeding potential of a sire.

Experiments were undertaken to develop, if possible, a fast, simple procedure which would minimize damage to the spermatozoa during freezing. Following conventional procedures for equilibrating semen for 5 to 6 hr. at 5° C. in a yolk-citrate-glycerol extender, samples were transferred abruptly to a freezing bath at temperatures of +5, 0, −5, −10, −15, −20, −25, −30, −35, and −40° C. The percentage of motile spermatozoa following freezing and storage for one, 30, and 60 days at −85° C. was higher when the samples were transferred from +5° C. to a freezing bath at −20 to −40° C., than when transferred to the bath at higher temperatures. The results of these studies show that the conventional slow procedure for freezing bovine spermatozoa may be speeded up by transferring the semen from +5° C. to a freezing bath ranging from −20 to −40° C., and freezing quickly to −75° C. Time and labor are saved, and the percentage of motile spermatozoa surviving freezing is increased.

OUR ASSOCIATION

THE 55TH ANNUAL MEETING OF THE AMERICAN DAIRY SCIENCE ASSOCIATION AT UTAH STATE UNIVERSITY, LOGAN JUNE 19-22, 1960

H. F. JUDKINS, Secretary-Treasurer
32 Ridgeway Circle
White Plains, New York

The attendance at the 55th Annual Meeting of the American Dairy Science Association was the second largest on record. It was a memorable meeting. The hosts were hospitable in the best Utah tradition. The weather was comfortable and the scenery beautiful.

The general session was held on June 21. In the absence of Dr. Daryl Chase, President of Utah State University, Vice-President M. R. Merrill extended greetings and welcomed the Association.

An informal get-together, which exuded hospitality, was held on Sunday evening in the Student Union building. On Monday evening, 1,800 people enjoyed a chicken barbecue in the amphitheater overlooking Logan, with the beautiful mountains beyond. Tuesday evening the Association and several hundred townspeople were entertained in the University field house by the famous Salt Lake City Tabernacle Choir. The voices of farmers, mothers, grandmothers, doctors, dentitsts, teachers, lawyers, and high school students thrilled the audience with hymns of contemporary and of sacred music. This event will be remembered for a long time.

On June 22, Secretary of Agriculture Ezra Taft Benson gave an excellent address to the Association.[1] This was followed by a symposium:[2] Facts, Fallacies, and Conflicts Affecting the Demand for Dairy Foods. W. D. Knox, Editor of Hoard's Dairyman, was the moderator. The speakers were Dr. M. R. Stephens of the Food and Drug Administration, Dr. Margaret A. Ohlson, State University of Iowa, and Dr. Zoe E. Anderson, Wayne State University. Interest was very lively and the discussion lasted until five o'clock.

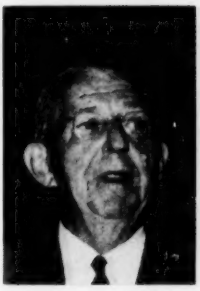
A number of student affiliate members were present and a Student Branch Section of the Association was formed.

THE PRESIDENT'S REPORT

A. C. FAY
12458 North Bay Shore Drive, Keystone Island
North Miami, Florida

THE HOSPITALITY OF OUR HOSTS

First, on behalf of the members of the American Dairy Science Association, I should like to express our sincere thanks to our host, the Utah State University, for the privilege they have extended in allowing us to be their guests on this beautiful campus. The warmth of your hospitality was evident several months ago from the cordial welcome extended to the Association by Governor George B. Clyde and by President Daryl Chase; later, by the willingness of Secretary Benson to grace our program and by the world-famous Salt Lake Mormon Tabernacle Choir to entertain us, and currently by the detailed plans and preparations for our comfort and entertainment worked out by Professor A. J. Morris and the members of his staff. Only by going through the mill himself can a person fully appreciate the vast amount of detailed planning, the many hours of hard work and sometimes sleepless nights, required to entertain a large convention such as this. Professor Morris, I want you to know that the members of this organization and their families deeply and sincerely appreciate the extraordinary job that has been done by you, the members of your staff, and by the members of other university departments who have cooperated with you.



Pres. A. C. Fay

THE WORK OF COMMITTEES

There are a great many people who should be thanked for their cooperation and effort during the past year. The amount of time taken from already busy schedules by more than 200 members and chairmen of the several committees of the Association well deserve individual mention. It is tempting to name all of these people, but obviously to do so would require more time than is allotted.

I am going to take the time, however, to single out and pay a well deserved tribute to two persons—our Editor-in-Chief and our Secretary-Treasurer.

THE WORK OF THE EDITOR-IN-CHIEF

Few people fully appreciate the detailed work required to put out our JOURNAL on time each month. When you or I have worked assiduously for long hours in the preparation of a manuscript, we heave a sigh of relief when we finally

[1] This address will be published.
[2] This symposium will be published.

mail our package of organized ambiguity to Dr. Herreid for publication. Little do we realize the major surgery and skin grafting involved in correcting the punctuation and spelling, the readjustments necessary to make our efforts conform to the style and format of the JOURNAL, and in my case—though not in yours—the elimination of contradictory statements and the spotting of unwarranted conclusions.

Voluminous correspondence is required between the Editor, the members of the Editorial Board who review the paper, and sometimes between the Editor and the author, in order to clarify some perfectly obvious point that any one should be able to understand if he were as confused as the author was when he wrote it. When the metamorphosis is finally complete and our article appears in polished form in the JOURNAL OF DAIRY SCIENCE, we view with pride "the excellence and clarity of our presentation." The American Dairy Science Association owes a great deal to Dr. E. O. Herreid and his staff of 16 associates on the Editorial Board.

APPRECIATION FOR SECRETARY JUDKINS

The other one person to be singled out for special commendation is our efficient and loyal Secretary-Treasurer, H. F. Judkins. I have already expressed to Dr. Judkins verbally and in writing my personal appreciation for the excellence of his work and his unswerving loyalty; hence, my purpose here is to give the members of this organization some idea of what Dr. Judkins is doing for the Association.

Without taking the time to list the items that make up the tremendous work load carried out by Jud, suffice it to say that it covers not only innumerable details and extensive correspondence, but also constant attention to the coordination of the activities of many committees and officers of the Association. He carries the responsibility for the financial management and personally solicits the advertising for the JOURNAL, without which the JOURNAL in its present form would be possible only by doubling the subscription price. Professional advertising agencies would not undertake this one job alone for a fee five times the small total pay which Jud receives from the Association. In fact, the so-called salary paid to Jud would not even pay the rent on the office space which Jud provides us free of charge. An organization is extremely fortunate to have among its members one whose devotion is so deep.

Jud, I don't know how to thank you with mere words. I do know that in industry such extraordinary service would be recognized by a substantial increase in pay, but you will not even allow us to do that. The nutritive value of applause is notoriously low; however, it may complement the inadequacy of nice words as an expression of our appreciation to you. Jud, I am going to ask you to stand while the members of this Association help me in expressing our thanks.

THE STUDENT BRANCH

Despite the limitation of time at my disposal, there are two items that have come into fruition during the past year which should be brought to your attention. Both of these have been in the incubation stage for several years, but have become a reality since our last meeting in Urbana.

The first harvest this year of the efforts of several preceding years is the birth of the new Student Branch of the American Dairy Science Association. This is the outgrowth of the work of our Student Affiliate Committee under the Chairmanship of Professor W. W. Snyder, and with the assistance of a special Sub-Committee headed by Professor Paul M. Reaves. This Committee has undertaken the very difficult task of building the foundation of this new Student Branch without the benefit of a precedent or pattern to follow. The organization is under way for the first time here at Logan, an event which may well be a milestone in the history of the American Dairy Science Association. To a very large extent the future of this Association will be found in the membership of this new Student Branch.

THE PLANT TRAINING MANUAL

The second fruit crop harvested this year from several years of previous effort is the publication of the Plant Training Manual. This may well be one of the outstanding contributions of the American Dairy Science Association to the processing plants in the dairy industry. The Committee, under the Chairmanship of Professor H. B. Henderson, has provided the industry with a practical tool for use in conducting a training course for promising employees who are marked for leadership. The organization of the Manual is such that it is applicable to the situation in small plants as well as in large plants, and is designed to fit the practical needs for plants handling any or all of the standard dairy products. The Plant Training Manual fills a need that has stymied the inauguration of training programs by many dairy companies. Since it is difficult for me to discuss this great contribution without seeming to be carried away with enthusiasm, may I suggest that you study it yourself and perhaps you will share that enthusiasm.

THE RESPONSIBILITIES OF THE AMERICAN DAIRY SCIENCE ASSOCIATION

The American Dairy Science Association is an organization of approximately 2,600 people in the United States and 11 other countries who are interested in the application of scientific methods to the various facets of the dairy industry. Many if its members are highly specialized in specific segments of the industry, be-

ginning with the grass lands on which the cows graze through to any one of the various finished forms in which dairy products are marketed.

The effectiveness of this organization depends upon how clearly we keep in focus the function we are expected to perform, and how keenly we are alert to the changing panorama of needs. At the risk of over-simplification, it seems that we are charged with three responsibilities: to train students, to conduct research, and through effective communications to promote the application of scientific methods to the dairy industry.

The organization is now divided into three main sections, Production, Manufacturing, and Extension. Two new groups have been added this year, the Student Branch already discussed and a subgroup of members interested in Extension in Manufacturing.

The Training of Students. The dairy industry looks to our colleges to train students for practical operating responsibilities in short courses, and also to provide training in basic fundamentals of dairy science in the four-year course and in graduate training. In the short courses, emphasis is placed on the operating procedures, with enough explanation of the fundamentals to give orientation. The objective is to provide industry with reliable operators who can intelligently perform the jobs for which they are trained.

Most ambitious young men who enter the four-year course, whether they recognize it or not, are actually hoping that some day they will occupy positions of greater responsibility than are required of routine operators. Their effectiveness will ultimately be measured by their ability to make sound decisions and to get work done through people. Adequate preparation involves not only an emphasis on basic principles upon which sound decisions may be made, but even more particularly upon learning the commonsense fundamentals of leadership.

Fortunate indeed is the young man whose college course gave him a thorough understanding of the fundamental principles of the biochemistry of nutrition, the physiology of reproduction, the physical principles and thermodynamics of heating and refrigeration, the electronics of automation, and many other basic concepts from which sound decisions may be made. In the coming 40 years of his active career methods will change with each new invention, but the basic fundamentals will change little if at all. It is far more important that he be thoroughly grounded in the chemical, physical, and biological processes involved in his profession than to know how to weigh out the feed for a cow, the technique of artificial insemination, or what valves to open and close in the operation of a refrigeration compressor or of a vacuum pan.

The Importance of Training in Leadership. If we are to keep alert to the needs of the rapidly changing pattern of the dairy industry, we should recognize that the consolidation of farms, herds, and processing plants, together with the increasing use of automation, will inevitably result in fewer operations and more management personnel. The young man who spends long and late hours mastering the basic principles of thermodynamics may be well advised to give equal attention to mastering the commonsense fundamentals of leadership. His opportunity for future success may depend more upon his ability to focus and harness the efforts of others than upon his technical proficiency.

The corner-stone of effective leadership is a superior knowledge of the field of interest, but this corner-stone must be supported by sincerity, unqualified honesty and integrity, loyalty to the group, patient tolerance, willingness to work, willingness to listen and advise, and above all, willingness to share the credit for success and to accept the blame for failure. These are the simple principles of getting along with people and inspiring a feeling of confidence. They involve the shifting of the focus of interest from one's self to that of one's associates.

A leader has been defined as anyone who has followers. The domineering type of leadership supported by fear is usually less effective than if supported by confidence and respect. It is a mistake to assume that the qualities of effective leadership are inherent and not developed in the individual. To ignite the spark of leadership will probably contribute more to the future success of a student than any technology he may learn in a given course. No opportunity should be lost to do so.

The dairy industry needs leaders and it is reasonable to expect that this need will increase. In the efforts to prepare students for a career in industry, training in the commonsense principles of leadership should be given high priority. Although consideration may well be given to including specific courses on the principles of leadership, it should be the ambition of teachers of all courses to inspire students for maximum service in the field of their choice.

The Research Program. The dairy industry also looks to our colleges and government agencies for research as the key to progress. Research may roughly be divided into two categories, sometimes called pure research and practical research. The line of demarcation between the two is not clear-cut.

Pure research may be illustrated by the work of a scientist who spends years exploring the metabolites of a microbial cell, with little or no concern for the possible utility of his findings. He is motivated by an irrepressible curiosity, and he gains his satisfaction from the fact that he has added to the store of scientific knowledge. If, many years later, some one picks up the fragment of this foundation and

develops a series of miracle drugs or antibiotics from his contribution, he is gratified but not particularly excited. A judicious percentage of research funds are and should continue to be allocated to the explorations of the cloudy areas of knowledge, with the hope of opening up completely new horizons of light.

The majority of research work conducted in our state and governmental experimental laboratories may be classified as practical research. Such studies are focused on imminent problems for which new knowledge is needed. The research worker is motivated by a keen desire to contribute to a complete or partial answer to the practical problem at hand. To use Mr. Judkins' terminology, he is not only a "producer" of research but he is sincerely hopeful that the "user" will find his contribution fruitful. His work may be just as fundamental as that of the pure research worker, but his target is more clearly defined.

The Importance of Communications. An essential feature of practical research is to have available effective means of communication so that the results may reach the "user." The American Dairy Science Association is well organized to provide these avenues of communication. The Extension Section, and now the new Extension group in Manufacturing, and particularly the JOURNAL OF DAIRY SCIENCE, are designed to bring the results of research to the attention of people who can use them.

Recently, a separate section of the JOURNAL OF DAIRY SCIENCE presents brief summaries of the technical articles in each issue. This is an attempt to make the JOURNAL useful to a larger number of readers. The JOURNAL necessarily covers the broad horizon of the entire dairy field. Its purpose is and always should be to publish truly scientific articles which contribute to the field of knowledge in all facets of the dairy industry. Although we try to keep abreast of progress in contiguous fields, few persons have the time or inclination to read all of the technical and statistical details in all of the articles in the JOURNAL. The new Summaries are designed to present the essential features of the research, thereby enabling better communications between the workers in the various segments of the industry. Workers in the fields of Production, Manufacturing, and Extension would like to be aware of the work in each other's fields, but do not have the time or, in some instances the background to interpret the details of the presentation. It should be kept in mind that over one half of our membership is made up of members from industry and are classified as "users" of research rather than as "producers" of research. This group profits particularly from the new feature of the JOURNAL. Comments are frequently heard that "the articles are over my head" or that "there are too many articles outside my area of interest." Such comments come not only from people in industry but also from highly specialized workers in other phases of dairy research. The practical fact is that in order to support a JOURNAL it is necessary to provide a service to a sufficiently large group to make it financially sound.

Secretary Judkins' report will show that the American Dairy Science Association has not only grown rapidly in numbers but, even more significantly, there has been a change in the pattern of membership. This can only reflect that the American Dairy Science Association has extended its area of service in a way that has made it attractive to an expanding group of people. The fact, however, that a considerable number of eligible people join our Association each year only to drop out after the first or second year indicates forcibly that we have failed to be of service to them. Many of these are the so-called "users" of research. We need not simply their financial support, but we need even more the opportunity which their membership affords to improve the communications between the "producers" and the "users" of research.

CONCLUSION

The American Dairy Science Association has played an extremely important part in the half-century of progress of the dairy industry, through the training of leaders and through conducting of valuable and timely research. Our contribution during the next half-century will depend on the extent to which we are alert in the adjustment of our training and research programs to meet the needs of the ever-changing demands of the times. The long-time contribution will depend on the extent to which our programs of pure research will open up new horizons of progress.

The time allotted for this part of the program has more than passed; however, I should be more than ungrateful if I did not express my very sincere thanks for the honor and privilege you have extended to me in allowing me to serve as your President during the past year. As we look forward to another year, under the leadership of Dr. Ralph Hodgson, I am sure we will be alert to the responsibilities and the opportunities for the American Dairy Science Association to serve humanity.

THE OPPORTUNITIES OF THE STUDENT BRANCH OF THE AMERICAN DAIRY SCIENCE ASSOCIATION

A. C. FAY, PRESIDENT
12458 North Bay Shore Drive, Keystone Island
North Miami, Florida

ORIGIN OF THE STUDENT BRANCH

For many years the American Dairy Science Association has endeavored to attract the in-

terest of students through Student Affiliate organizations in our various colleges and universities where dairy technology is taught. A substantial percentage of the present membership of the American Dairy Science Association is made up of persons who have been members of Student Affiliate chapters during their school years. Although the organization of student groups is necessarily a local matter, the Student Affiliate Committee of the American Dairy Science Association has served as a focal point in the promotion and encouragement of these groups of young dairy scientists.

It was recognized several years ago, however, that there was need for an organization structure which would more effectively mold the common interests of these student groups with those of the American Dairy Science Association. Accordingly, the Student Affiliate Committee was assigned the task of exploring the problem and submitting a proposed plan to fill this need.

The meeting you are attending today is the outgrowth of many months of work and several years of planning to formulate an organization of Student Affiliate chapters into a Branch of the American Dairy Science Association. It is hoped that by giving these chapters a national status, and by focusing their efforts toward a common goal, it will strengthen both the parent organization and the individual chapters.

It is appropriate to take time here to pay tribute to the members of the Student Affiliate Committee, under the chairmanship of Professor W. W. Snyder of Michigan State, and the members of the Sub-Committee, under the chairmanship of Professor Paul M. Reaves of Virginia Polytechnic Institute, with the assistance of Dr. A. W. Rudnick Jr. of Kentucky, Dr. J. B. Mickle of Oklahoma, and Dr. J. B. Williams of Minnesota.

Since there were no patterns or precedents to follow, these men had the difficult task of building the foundation and skeletal structure for this new branch, with imagination, foresight, and sound judgment as their only guide. It was not intended that they should hand you men a completed organization with all of the details worked out. Rather, it seemed preferable to provide you with a sound foundation and the framework about which you could design a structure of your own choosing. In this organization meeting it is hoped that there will emerge the leadership and the vision to complete the architectural details in a fashion that will make the final structure one of your own design. Although Professor Snyder and Professor Reaves and his Sub-Committee will work closely with you, it is their desire and hope that when the new organization is completed it may truly be regarded as a product of the students themselves.

OBJECTIVE OF THE STUDENT BRANCH

There are five essential features of a successful organization.

(1) It must have a clearly defined objective
(2) It must fill a need
(3) It must render a service to its members
(4) It must be financially sound
(5) It must have strong leadership.

It is neither my purpose nor my prerogative to provide the answers to these essentials. I am convinced, however, that there is a need for an organization that will focus the efforts of widely scattered student groups toward a common goal. That goal should be to promote the application of scientific methods in all phases of the dairy industry, to attract more high-caliber students to the field of dairy technology, to adjust the contents of courses and of curricula to the rapidly changing structure of the industry, to improve the teaching methods for preparing young men for a life-time of constructive service, and to serve its members by providing the platform and the communications for the exchange of ideas in the field of dairy research.

Although it is right and proper that the major interests of local chapters should be centered on the local situation, there is much that widely separated groups can do by combining their efforts on problems of mutual interest. A recommendation from a national organization of dairy students on matters pertaining to methods of recruitment, better courses, improved teaching methods, etc. would carry more prestige and weight than if it originated in a local chapter.

From the viewpoint of intelligent self-interest, the American Dairy Science Association is interested in promoting this organization, not only for the constructive suggestions and help it may receive but also as a training ground for future leadership. Participation in committee activities and in the official positions of the Student Branch of the American Dairy Science Association will provide a broad background of experience for future responsibilities in the Association or in industry.

ORGANIZATION OF THE AMERICAN DAIRY SCIENCE ASSOCIATION

The business affairs of the American Dairy Science Association are handled by a Board of eleven persons, six of whom are elected for a three-year term on a rotating basis, two retiring and two replacements each year. In addition to the six elected members, the President, the Vice-President, the Immediate Past-President, the Secretary-Treasurer, and the Editor of the JOURNAL OF DAIRY SCIENCE serve as ex-officio members of the Board. The Secretary-Treasurer and the Editor of the JOURNAL are elected by the Board each year.

The membership elects a new Vice-President each year, who automatically becomes President of the Association the following year. It is customary for the candidates nominated for Vice-President to be selected on alternate years from persons representing Production and Manufacturing, and occasionally to give the membership a choice of candidates representing industry. Similarly, the choice of candidates for replacing the two retiring members of the Board are made in such a way as to keep the balance between Production, Manufacturing, Extension, and Industry.

For the purpose of segregation of interests, the American Dairy Science Association is currently divided into three broad sections, Production, Manufacturing, and Extension. Each section has its own Chairman, Vice-Chairman, and Secretary. The Secretary of the section elected each year automatically becomes the Vice-Chairman the following year, and Chairman of the section the third year. Although each section sets up its own program for the Annual Meeting, the three Section Chairmen are ex-officio members of the General Program Committee, which in turn is responsible for coordinating all of the features of the over-all program.

This year two new groups have been added, one of which is the Student Branch; and another group interested in Extension in Dairy Manufacturing has developed a separate program.

Each section has its own Sectional Committees assigned to study and report on matters pertinent to the interests of the section.

For the organization as a whole, there are eleven standing committees and seven Awards Committees. Most of these committees have a rotating membership, with two members retiring and two new ones appointed each year, thus providing continuity of policy and at the same time introducing new thinking into the committee.

There are seven major awards granted through the Association each year for outstanding achievement in the various fields of Production, Manufacturing, Extension, and Teaching.

The outstanding project of the American Dairy Science Association is the publication of the JOURNAL OF DAIRY SCIENCE, which is recognized throughout the world as one of the best in its field. It is devoted to the publication of original reports of fundamental and practical research in all phases of dairy technology. The JOURNAL OF DAIRY SCIENCE is now in its 43rd year of publication and may be found in most of the libraries of the world carrying technical journals.

There are approximately 2,600 members of the American Dairy Science Association, over 200 of whom are to be found in eleven countries in all parts of the world, including Australia, Asia, Africa, and many others.

The facts that the American Dairy Science Association is now in its 55th year, that it has grown rapidly, not only in numbers but in prestige, that it is recognized widely throughout the world, can only be explained in the two words "service rendered." As you proceed in the formulation of this new Student Branch it would be well to consider that the length of its existence will be measured in terms of the same two words, "service rendered." The need is there and the opportunity to serve is ample. If the architectural design and the craftsmanship are good, if the vision and judgment employed are sound, I am sure that in years to come those of you in this room will look back to this day and say with pride—"I helped in starting that organization."

THE STUDENT BRANCH

Under the leadership of Professor Paul M. Reeves of Virginia Polytechnic Institute, Blacksburg, Va., a Student Branch section of A.D.S.A. was organized.

Three sessions were held as scheduled; the first one for organization and appointment of committees; the second included committee reports and student discussion. The Constitution as presented by the committee was approved.

At the last session, Mr. Ray Dankenbring, one of the judges of the Chapter exhibits, presented an interesting review of the exhibits.

Officers of the section were elected as follows:

President, Lewis D. Meyer (Michigan)

First Vice-President, Joseph A. Lineweaver (Virginia)

Second Vice-President, Robert Van Pelt (Washington)

Third Vice-President, Marvin E. Oetting (Missouri)

Secretary-Treasurer, Francis Rothauge (Oregon)

The first officers of the section then met with the organization committee and discussed plans for the 1961 meeting.

AWARDS PROGRAM

The awards program was held on the evening of June 21 in the University auditorium. Professor J. J. Barnard, Extension Dairyman, presided over the entertainment. He introduced Andrew Galos, violinist; George Pahtz, celloist; and Norma Roberts, pianist. This group delighted the audience by playing the Serenade by Gabriel Pierné, the Melody by Peter Tschaikovsky, and Bolero by Moritz Moszkowski.

Dr. A. C. Fay presided over the awards ceremonies, and he responded with appropriate

remarks as the names of the honored students and scientists were announced by chairmen of the various Awards Committees.

John A. Jaynes, graduate student at the Michigan State University, won the Graduate Student Scientific Paper award in manufacturing and was presented with a check for $100, and an appropriate certificate from the Association by Dr. W. F. Shipe of Cornell University. J. T. Huber, graduate student at Iowa State University, won the Graduate Student Scientific Paper award in production and was presented with a check for $100 and suitable certificate from the Association by Professor J. E. Johnston of Louisiana State University.

The Virginia Polytechnic Institute Student Chapter was judged as having the best exhibit of its activities and was awarded a check for $100 and a plaque from the Association by Professor W. W. Snyder of Michigan State University, who is Chairman of the Student Affiliate Committee.

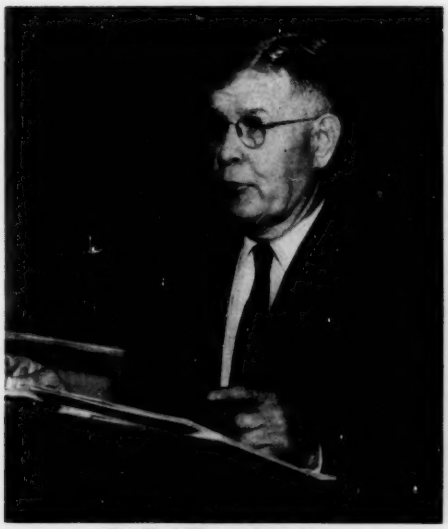
Dr. W. F. Shipe, with President A. C. Fay, presenting the American Dairy Science Association Scientific Paper Award in Dairy Manufacturing to John A. Jaynes of Michigan State University.

Professor J. E. Johnston, with President A.C. Fay, presenting the American Dairy Science Association Scientific Paper Award in Dairy Production to J. T. Huber of Iowa State University.

Professor W. W. Snyder, with President A. C. Fay, presenting award to Joseph A. Lineweaver of Virginia Polytechnic Institute for the most outstanding exhibit.

Citation by Professor A. J. Morris for the Recipient of the American Dairy Science Association Teaching Award in Dairy Production for 1960

William Lyons Phelps, the late distinguished teacher of Yale University, once said: "Teaching is an art—an art so great and so difficult to master that a man or woman can spend a long life at it, without realizing much more than his limitations and mistakes, and his distance from the ideal." Nevertheless, the teacher of dairy producton selected for special recognition this year by the American Dairy Science Association has come a long way in mastering that art. For the most part, his academic fields lay in the great Mississippi Valley. Here, he plowed well, straight, and deep.



Dr. Earl Weaver lecturing.

His pedagogical furrows brought to light and development talents where often none seemed to exist. The products of his classroom are to be found throughout the world. Among them are men of prominence and distinction, including college teachers, university presidents,

experiment station workers, editors, legislators, chairman of industrial boards, association secretaries, manager of dairy enterprises, farmers —in fact, leaders in all walks of life.

Few teachers in a lifetime have projected themselves into the lives of others as the chosen recipient of the Teaching Award for 1960. Not only could he perceive latent possibilities in students but instilled into them a desire to give only their best. He possesses that rare aptitude of being able to sublimate himself into the life of each individual student with whom he comes in contact. Through former students, we sense him in the classrom. They recalled:

"No class of his was ever a sea of faces. Each student was an individual, was treated as such, and each was stimulated to develop to the maximum of his individual capacities. Even in class the student felt that he was being spoken to personally rather than as a member of the class."

"Nothing less than 100 per cent satisfied him when the honesty, integrity, or the effort capacity of the student was involved."

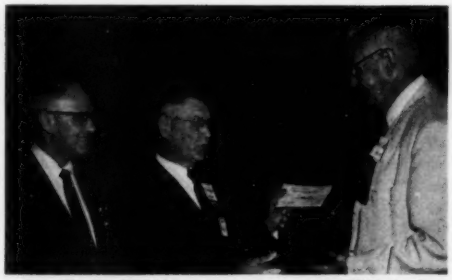
"He inspired people to wish to accomplish."

"He seemed to have a 'sixth' sense for sizing up students and guiding them into proper channels for their life work."

Early in his career he distinguished himself as a highly successful coach of dairy cattle judging teams. On one occasion he accompanied his championship 4-H dairy cattle judging team to England and Europe, where it represented the United States in the classic dairy cattle judging contest.

Following service in World War I as a marine, he taught agriculture to soldiers of the American Expeditionary Force in France. Again, after World War II, he taught the soldiers dairying at Shrivenham American University, England, at the request of the United States Military forces. Very recently, he was selected by his university to administer its Colombia, South America, Point-4 program, serving his country four years, establishing and teaching the first dairy manufacturers short course ever held in Colombia.

In addition to achievements of the classroom, the selectee for the Teaching Award for 1960 is a writer of note, having authored or coauthored two books, over 100 technical papers and research bulletins, and innumerable popular articles. Never forsaking the classroom, which he first entered as an associate professor at the University of Minnesota in 1917, he demonstrated also real ability as an administrator. Successively, he was in charge of the dairy husbandry section at Iowa State University, and headed the dairy departments at Oklahoma State University and Michigan State University —the latter for 18 years. Not surprising, he was early included in "Who's Who in America."



Dr. H. F. Judkins representing the Milk Industry Foundation, presenting the American Dairy Science Association Teaching Award to Dr. Earl Weaver, with Professor A. J. Morris, who read the citation.

Despite the demands of teaching, research, and administration, he took time to serve the American Dairy Science Association as a loyal member for over two-score years; meanwhile, he served on various committees and later as a Director, Vice-President, and President.

The recipient for the Teaching Award in Dairy Production for 1960 was born near Brazil, Indiana, reared in Oklahoma, for the most part received his elementary and high school training in Oklahoma, baccalaureated at Oklahoma State University, mastered at Iowa State University, and earned the Ph.D. degree at the University of Minnesota. Will Dr. Earl Weaver please come forward?

President Fay, on behalf of the Teaching Award Committee, it is my pleasure to present to you Dr. Earl Weaver of Michigan State University who, for two-score years of distinguished teaching, has been selected to receive the Teaching Award for 1960.

Citation by Professor T. C. Blalock for the Recipient of the DeLaval Extension Award for 1960

The recipient of the 1960 DeLaval Extension Dairyman's Award has devoted his entire life to the service of the people of his home state of Wisconsin.

He was born in 1898 and began his public service career as an Official Test Supervisor for his State University in 1920. From 1923 to 1939 he was associated with the University Dairy Herd, starting first as a milker, then as foreman, and as chief herdsman for the last eight years of this period. As chief herdsman, he was closely associated with and played a vital part in the University's dairy research program.

While working with the University herd, he was also able to complete the requirements for a Bachelor of Science Degree in Dairy Production in 1933. In 1939 he accepted a position with the University as Extension Dairyman, which he has ably filled. He is currently

Prof. G. M. Werner explaining hay quality.

serving as Chairman of the Extension Production Committee.

While he has made significant contributions in all phases of the dairy extension program, he is best known for his leadership in the fields of artificial breeding and improved roughage quality. One of his first assignments in 1939 was to assist in the development of the infant artificial breeding program in his native state. Evidence of the success he enjoyed is the fact that today this state is recognized as the leader in this field. As this breeding program grew and under his guidance local leaders began to assume more responsibility for its operation, he began to direct his attention to the problem of improving the quality of roughage on dairy farms. In 1949, he organized a series of roughage clinics that have become annual events and have been held in every county in the state. He was instrumental in organizing a state-wide grassland farming contest that has involved more than 3,000 dairy farmers. His Milk Per Acre demonstrations on approximately 250 farms each year have provided tangible proof of the value of pastures and good pasture management.

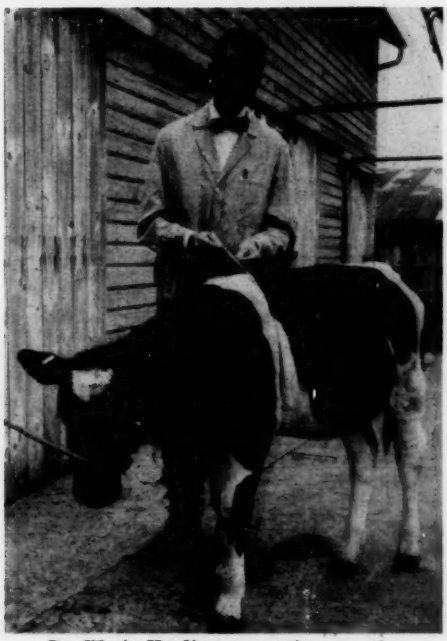
Mr. Thomas Braddock of the DeLaval Company presenting the DeLaval Extension Dairyman Award to Professor G. M. Werner, with Professor T. C. Blalock, who read the citation.

He is a member of Alpha Zeta and has served as Director and President of his State Extension Workers Association. He is currently a member of his Governor's Advisory Committee on Dairying and has served on University committees too numerous to mention. He has been a member and served as Chairman of the Dairy Cattle Health Committee of the American Dairy Science Association as well as a member of the Journal Management Committee. During 1954, 1955, and 1956, he served as Secretary, Vice-Chairman, and Chairman of the Extension Section of the annual meetings and as a member of the Board of Directors of this Association from 1956 to 1959.

Born in New London, educated at the State University, and for forty years a faithful public servant of the dairy farms of the great dairy state of Wisconsin—Mr. President, it gives me great pleasure to present Professor George M. Werner from the University of Wisconsin as the recipient of the 1960 DeLaval Extension Dairyman's Award.

Citation by Dr. J. F. Sykes for the Recipient of the American Feed Manufacturers Award for 1960

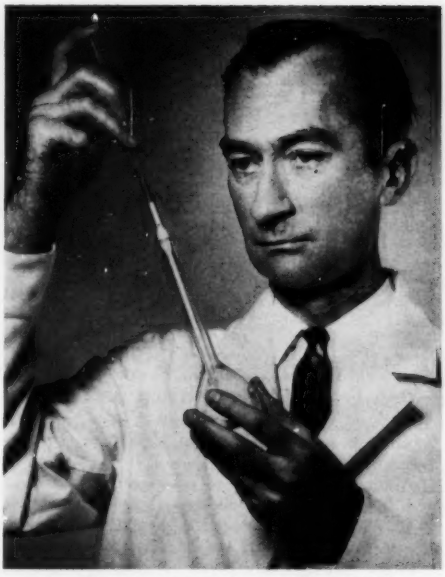
The recipient of the 1960 American Feed Manufacturers' Award for 1960 was born in Barren County, Kentucky, in 1925. He re-



Dr. W. A. Hardison measuring growth.

ceived the Degree of Bachelor of Science in Agriculture from Western Kentucky State College in 1947. His Master of Science degree was received in 1949 from the University of Kentucky and in 1952 he earned his Ph.D. degree at Cornell University. Since 1952 he has been a staff member at Virginia Polytechnic Institute, where he is Associate Professor of Dairy Husbandry. He is a member of Alpha Zeta, Sigma Xi, the American Dairy Science Association, and the American Society of Animal Production.

The recipient has made significant contributions to dairy cattle nutrition through his studies on copper deficiency and metabolism in calves and on the effects of rumen inoculation and frequency of feeding in growing heifers. He has also conducted work on feeding arsenicals to calves and on the protein requirements of the young calf. Another major interest has been the development and use of ratio techniques for determining the nutritional value of forages, and studies on factors which affect the useful application of these techniques. He has contributed materially to the extensive forage program at his institution. In 1958 his contributions to forage evaluation were recognized by an invitation to present a paper entitled, Evaluating the Nutritive Quality of Forage on the Basis of Energy—A Review, before the joint meeting of the American Grassland Council and the American Dairy Science Association.



Dr. Mark Keeney making milk analysis.

Mr. W. T. Diamond presenting the American Feed Manufacturers Award to Dr. W. A. Hardison, with Dr. J. F. Sykes, who read the citation.

The man of whom I have been speaking will by now be recognized by many of you as Dr. W. A. Hardison.

Citation by Dr. E. O. Herreid for the Recipient of the Borden Award in Dairy Manufactures for 1960

The recipient of the Borden Award in Dairy Manufactures this year was born May 18, 1921, in Sharon, Pennsylvania. He was raised on a dairy farm in Essex County, New Jersey, and attended the public schools in Cedar Grove and Bloomfield.

He earned the Bachelor of Science degree, with a major in Dairy Husbandry, at Pennsylvania State University in December, 1942. From January, 1943, to August, 1944, he was employed as a chemist in the Special Products Division of the Borden Company. He served as an officer in the U. S. Navy from August, 1944, to October, 1946. Immediately after his discharge from the Navy, he enrolled in the Graduate School of The Ohio State University and earned the M.S. degree in Dairy Technology in August, 1947. In June, 1948, he returned as a graduate student to Pennsylvania State University, where he earned the Ph.D. degree in 1950.

This young man's early interest in Dairy Science was obtained on his father's farm. His outstanding contribution was the development of a column chromatographic method for butyric acid. This method has been adopted by the Association of Official Agricultural Chemists for measuring butyric acid in fat. It has also been used very effectively in studying the acids in silage and it has helped to reveal the structure of milk fat. Other useful research contributions include chemical studies of milk fat oxidation, cheese flavor development, the browning reaction in milk products, detection of milk fat adulteration, and development of column chromatographic procedures for separating and identifying carbonyl compounds.

Many organizations claim this young man as a member, including Sigma Xi, Gamma Sigma Delta, American Association for the Advancement of Science, American Chemical Society, New York Academy of Science, and the American Dairy Science Association. He is a consulting chemist to the Agricultural Research Service in the United States Department of Agriculture.

Mr. J. H. Erb presenting the Borden Award in Dairy Manufacturing to Dr. Mark Keeney, with Dr. E. O. Herreid, who read the citation.

His interests have not been confined entirely to scientific pursuits. During his undergraduate and graduate days he became interested in bridge and golf. In bridge circles he is well known as an early advocate of the psychic bid and one of the first to make such bidding unpopular. On the golf course he is known as the person who can throw a nine iron the farthest and is a good golfer in the low eighties. He is also strongly attracted to equine race and field events.

The recipient's father was an internationally famous breeder of Holstein cattle. He has two brothers who also earned the Ph.D. degree at Pennsylvania State University and both occupy prominent positions. We are honored tonight by the presence of his mother.

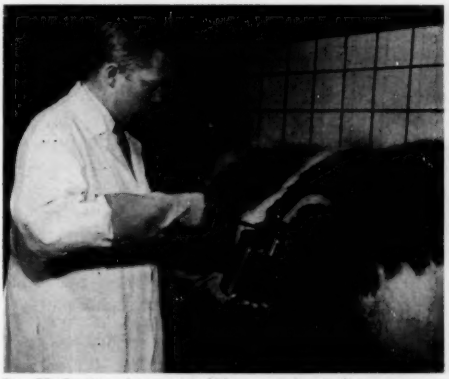
Our honored scientist comes from the outer circles of the nation's capital and we are happy to extend our sincerest congratulations to Dr. Mark Keeney of the University of Maryland.

Citation by Dr. I. R. Jones for the Recipient of the Borden Award in Dairy Production for 1960

The person selected as the recipient of the Borden Award in Dairy Production has made an outstanding reputation, not only as a teacher and research worker but also as a public speaker before farm groups, feed industry people, and groups of technicians in his own and related fields. He has been especially cited by his colleagues for the excellent manner in which he handles the efforts of graduate students and for the way in which he brings together associates in his own and different departments, so as to develop an effective team approach in solving complex problems relating to the dairy cow.

Specifically, the person selected has made notable research contributions, in cooperation with a group of scientists in related fields, in studying the interrelationships between rumen fluid characteristics and bloat pathogenesis, the relationship of bloat to plant species and composition, and the effectiveness and practicability for bloat control of a number of agents, especially detergents, antibiotics, and various oils. Other studies have been concerned with the effects of feeding insecticide-treated alfalfa hay and trichloroethylene-extracted soybean oil meal.

Calf nutrition studies in which he has been involved include normal aspects of rumen development and digestion, the relation of digestive enzymes to different feed additives, the increased bone growth of chlortetracyline-fed calves, utilizing Ca^{45} and radio-autograph techniques, the reduction in the incidence of diarrhea in calves fed milk fat, the value of corn distillers dry solubles in milk replacers, and factors which affect carotene and vitamin A assimilation from the alimentary tract. Other calf studies have been concerned with blood hemoglobin and with reducing sugar levels.



Dr. N. L. Jacobson obtaining specimen from rumen.

Another field of study in which he has cooperated with associates in meritorious contributions concerns the effects of various hay-concentrate ratios and feeding levels as measured by digestibility determinations and performance patterns of milk cows. With the development of production functions from the basic data, the investigators have presented surface maps and tables to use for predicting responses to different input-output quantities.

He was born September 11, 1918, at Eau Claire, Wisconsin. He received his B.S. degree at the University of Wisconsin in 1940 and his M.S. degree at the Iowa State University

in 1941, where he also obtained his Ph.D. degree in 1947. During the period from 1942-1946 he served in the U. S. Navy. He has been on the faculty at Iowa State University since 1947 and at the present time is Professor in Charge of Dairy Nutrition. He has received many academic and professional honors, including membership in Sigma Xi, Phi Kappa Phi, Alpha Zeta, Gamma Sigma Delta, and having been selected as a co-recipient of the American Feed Manufacturer's Award in Dairy Nutrition in 1955. He has been an active member of the American Dairy Science Association, being Chairman of the Production Section in 1959, and is Chairman of the A.D.S.A. Program Committee for 1960. He is a member of the Editorial Board of the JOURNAL OF DAIRY SCIENCE.

Mr. J. H. Erb presenting the Borden Award in Dairy Production to Dr. Norman L. Jacobson, with Dr. I. R. Jones, who read the citation.

On behalf of the Dairy Production Borden Award Committee, it is my pleasure to present Dr. Norman L. Jacobson of the Animal and Dairy Husbandry Department of Iowa State University, as the recipient of the 1960 Borden Award in Dairy Production.

Citation by Dr. A. C. Dahlberg for the Recipient of the Paul Lewis Award in Cheese Research

The recipient of the Paul Lewis Award in Cheese Research for 1960 is a native of Connecticut. He attended schools in that state and received the B.S. degree from its University in 1939. He began graduate work under a Dairy Industry Supply Association Fellowship at Cornell University, where he was granted the degree of M.S. in 1941 and the Ph.D. in 1944.

While attending college, he worked summers in a milk plant and he was an instructor in Cornell University during his graduate work. In 1952, he was the Guest Speaker from the United States on cheese research at the United Nations Technical Meeting in France and was United States Observer at the annual meeting of the International Dairy Federation meeting in Spain. He received a Fulbright Research Scholarship Award for research and lectures in France in 1954; The Borden Award for Research in Dairy Manufactures in 1955; and was a Fulbright Research Fellow in Ireland in 1959. He was made an Honorary Member of the Veterinary Science Society of Italy in 1956. He is a member of the committee on antibioties for the American Dairy Science Association.

Dr. F. V. Kosikowski in his laboratory.

His research applies specifically to many aspects of fundamental science and basic practices of cheese making. His outstanding book, Advances in Cheese Technology, was prepared in collaboration with Dr. Germain Mocquot of France and published by the Food and Agricultural Organization of the United Nations. His research on a special strain of bacteria for use as a Cheddar Cheese starter and his association of specific compounds with flavor in ripened cheese were noteworthy for the new knowledge revealed and for stimulating general interest among research workers in this vital field of study. His scientific contributions have improved the process of manufacturing Cottage Cheese. His studies have broadened our knowledge of the phosphatase test and he developed a special method for testing cheese. Of im-

Mr. Vincent Bavisotti presenting the Paul Lewis Award in Cheese Research to Dr. Frank V. Kosikowski, with Dr. A. C. Dahlberg, who read the citation.

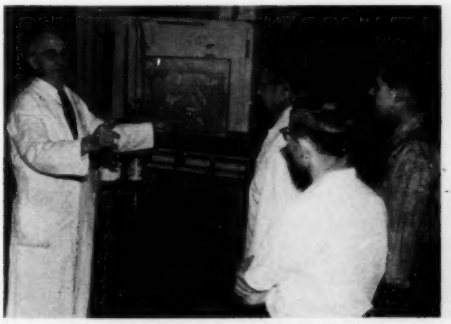
mediate concern is his adaptation of the disk assay method into a rapid, practical procedure of much promise, which is an outgrowth of his studies on natural bacterial inhibitors and antibiotics in milk. The authorship of the contributions shows a liberal spirit of sharing credit with his associates.

It is a special pleasure for me, on behalf of the Awards Committee, to announce that the recipient of the Paul Lewis Award in Cheese Research for 1960 is Dr. Frank V. Kosikowski, Professor of Dairy Industry at Cornell University.

Citation by Dr. C. F. Huffman for the Recipient of the American Dairy Science Association's Award of Honor for 1960

The Award of Honor recipient for 1960 was born on a farm in Minnesota. The large number of cows that he had to milk by hand while attending high school provided him with an early insight into the dairy business.

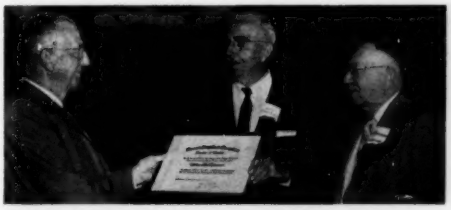
He received the B.S. degree in Dairy Husbandry at the University of Minnesota in 1916 and the M.S. degree in 1917 at the same institution. After spending three years as a dairy extension specialist at Kansas State University, where he was active in developing the early dairy extension program in that state, he returned to Minnesota, where he spent one year as fieldman for the Minnesota Holstein-Friesian Association. In 1921 he joined the staff of the Dairy Department of the University of Minnesota and received his Ph.D. from this institution in 1928. Since 1940 he has held the rank of Professor in the Dairy Department of the University of Minnesota.



Dr. W. E. Petersen lecturing.

He is recognized nationally and internationally for his research. Although broad in scope, his research has been directed more particularly toward milk secretion. His early major contributions include a refinement of the Babcock test, and a study of artificially induced hypoglycemia and hypocalcemia and their relation to milk fever. His mastitis studies have led to improved management practices and milking techniques which have saved the dairy industry millions of dollars. His fundamental research on the let-down process in milk production has been a boon to the dairy industry.

Probably his greatest contribution to dairy science has been his research on the mode of synthesis of milk constituents by the mammary gland, using the perfusion technique. At the present time he is investigating the production of antibodies by the mammary gland and their absorption from milk in the human digestive tract.



President A. C. Fay presenting the Award of Honor to Dr. W. E. Petersen, with Dr. C. F. Huffman, who read the citation.

He has been invited to lecture in many foreign countries, including England, Australia, and New Zealand.

He received the Borden Award in 1942, the first Teaching Award in Dairy Production in 1956, and the Morrison Award in 1956. He is author of the standard college textbook, Dairy Science, and is co-author of six other textbooks on agriculture and has also produced two motion pictures dealing with the science of milk production. He also received the Knight Order of Dannebrog from the King of Denmark.

He has served the American Dairy Science Association as a member of the Editorial Board, Abstract Editor, Director, and Vice-President. He was President in 1949.

Therefore, because of these outstanding contributions to dairy science and to the American Dairy Science Association, Dr. William Earl Petersen has been selected as the 1960 recipient of the highest recognition of our Association—The Award of Honor.

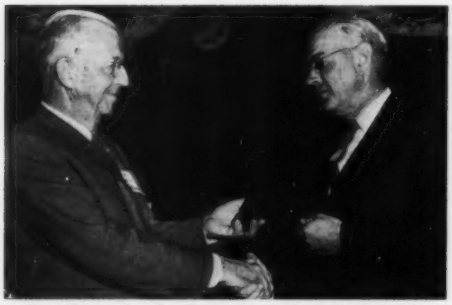
INSTALLATION OF OFFICERS

President A. C. Fay installed the following officers: S. T. Coulter and J. T. Reid as Directors; E. L. Jack, Vice-President; and R. E. Hodgson, President.

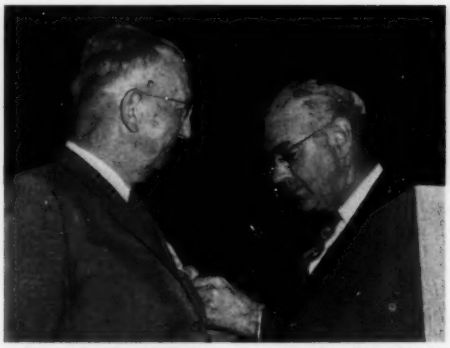
President Fay then presented the gavel to President-Elect Hodgson, who pinned the Past-President's ribbon on Dr. Fay and the meeting was adjourned.

President A. C. Fay installing S. T. Coulter as Director. J. T. Reid was in Europe.

Vice-President E. L. Jack receives instructions and the A.D.S.A. Handbook from President A. C. Fay.



President A. C. Fay presenting the gavel to President-Elect R. E. Hodgson.



President R. E. Hodgson pinning the Past-President's ribbon on Dr. A. C. Fay.

PAST-PRESIDENTS DINNER

The Past-Presidents dinner has become a regular event and it was held on June 21. Fifteen out of 29 past-presidents and members of the Executive Board were present. Before the dinner, the subject, The Interest of the Association in International Dairy Science Activity, was discussed. It was emphasized that the foreign membership of the Association had grown to more than 100 members, exclusive of Canada, which also has about 100 members. Furthermore, the number of foreign students being trained in American universities is increasing. The speakers, G. M. Trout, I. A. Gould, E. L. Jack, and K. L. Turk, and the discussants, agreed that these were desirable trends. Ways were emphasized to extend the influence of the Association in foreign lands and to recognize the different cultures of people in these lands, in order to successfully cooperate with them for the mutual benefit of all concerned. President A. C. Fay presided.

GENERAL BUSINESS MEETING OF THE ASSOCIATION

PRESIDENT A. C. FAY, presiding

The general session devoted to Association affairs was held on June 21. Vice-President

Past-Presidents A.D.S.A. Sitting: C. F. Huffman, R. B. Becker, R. Graves, Earl Weaver, Fordyce Ely, K. L. Turk. Standing: D. V. Josephson, H. B. Henderson, I. A. Gould, A. C. Dahlberg, G. M. Trout, W. E. Petersen, J. A. Nelson, H. F. Judkins, W. V. Price.

Dr. H. F. Judkins, Secretary-Treasurer; Dr. A. C. Fay, President; and Dr. R. E. Hodgson, Vice-President, in the shadow of Old Main Tower, Utah State University.

R. E. Hodgson summarized the action taken by the Executive Board on the various committee reports. Secretary H. F. Judkins and Editor E. O. Herreid gave their reports. Both reports will be published in the JOURNAL.

The Association presented 14 resolutions by V. H. Nielson, Chairman of the Resolutions Committee.

Brief reports were presented by Extension, Manufacturing, and Production section chairmen.

W. D. Knox, President of the Dairy Shrine Club, explained its activities and the advantages of membership in this club.

Dr. A. C. Fay, in his scholarly way, gave the presidential report.

MEETINGS OF THE EXECUTIVE BOARD

The subcommittees of the Executive Board met on June 17 to consider the various committee reports which had been prepared. This made it possible for the Executive Board to act more efficiently on Association affairs. There was an unusually large volume of old and new business and committee reports to consider on June 18 and 19 and briefly again on June 21. The Executive Board actions are presented in the summary that follows.

1. *Minutes of 1959 Board Meeting.* It was voted that the reading of the minutes of the 1959 meeting presented by the Secretary be eliminated.

2. *Results of certain 1959 Board meeting actions.* The Secretary reported on the activation of decisions made at the Board meeting of 1959.

3. *Report on interim actions.* Voted to confirm the Secretary-Treasurer's report on actions taken by mail since the 1959 meeting.

a. Codification of Board minutes brought up to date. This has been done and is very helpful.

b. Student Branch amendment. This amendment has been passed overwhelmingly. This provides (1) for submitting Journal reports of local student branches to the Secretary-Treasurer of the Association by June 1; (2) the establishment of a National Student Branch of the Association.

c. Election of life members. The following have been elected to lifetime membership effective January 1, 1960: T. V. Armstrong, Columbus, Ohio; P. T. Dix Arnold, Gainesville, Florida; A. H. Bayer, Schenectady, New York; G. B. Caine, Logan, Utah; Mason Campbell, Kingston, Rhode Island; V. O. Dreyer, Dayton, Ohio; T. W. Gullickson, St. Paul, Minnesota; P. S. Lucas, East Lansing, Michigan; E. J. Perry, Highland Park, New Jersey; A. H. Robertson, Delmar, New York; H. A. Ross, Santa Barbara, California; A. W. Rudnick, Ames, Iowa; P. H. Tracy, De Land, Florida.

d. Election results: The following officers were elected for 1960-61: R. E. Hodgson, President; E. L. Jack, Vice-President; J. T. Reid and S. T. Coulter, Directors.

4. *Election of new life members.* The following were elected to Life Membership effective January 1, 1961: A. C. Ragsdale, University of Missouri; C. A. Hutton, University of Tennessee; W. H. Martin, Kansas State University; and Stanley J. Brownell, Cornell University.

5. *Garrard Press Contract.* Voted that the request for an 8 per cent increase in the cost of printing the Journal be granted, effective July 1, 1960.

The Executive Board in action. R. E. Hodgson, E. L. Jack, N. P. Ralston, G. H. Wise, F. J. Doan, K. L. Turk, H. A. Herman, E. C. Scheidenhelm, E. O. Herreid, Sally Hunsaker, H. F. Judkins, A. C. Fay.

6. *Dairy Science Abstracts (English) for 1961.* Voted that the abstracts be continued for another year under the present contract of 750 copies at $4.90 per copy. Voted that a committee, consisting of the President, Vice-President, and Editor, including the A.D.S.A. representative to the National Research Council, be requested to keep in touch with the developments in abstract services.

7. *Acceptance of future award offers.* Voted that the Board accept the awards offered by the following companies for 1961: Milk Industry Foundation, The Borden Company, The DeLaval Separator Company, The American Feed Manufacturers, and The Paul Lewis Laboratory, and that the Secretary be directed to extend the thanks of the Association and of the Board to these companies and foundations for their continued interest and support of the awards program.

8. *Dairy Manufacturing Extension Group.* Voted to recommend to the Program Committee that a representative from the Dairy Manufacturing Extension Group be invited to participate in actions of the Program Committee.

9. *Future meeting dates.* The following dates have been confirmed: In 1961, the A.D.S.A. will meet in Wisconsin, June 11-14; 1962, Maryland, June 18-20; 1963, Purdue, June 17-19; 1964, Georgia; 1965, Kentucky.

10. *Registration fees at Annual Meeting for Award of Honor and Life Members.* Voted that the procedure previously followed, whereby life and honorary members pay their own annual meeting registration fees, be continued.

11. *Registration fees for dairy staff of host institution.* Voted that the matter of registration fees for members of the dairy staff at the host institution be decided by the host committee.

12. *Dairy Economics Section survey.* Discussion: Twenty schools were surveyed as to (a) whether there was a need for such a section; (b) whether the need for a group of dairy economists was being satisfied in present organizations; (c) whether, if such a section was set up, it would induce them to join the Association. An analysis of a survey of 55 persons showed 25 in favor of a Dairy Economics Section, 19 doubtful, and 11 opposed.

Voted not to appoint a committee to pursue the matter further, but directed the Program Committee to consider the possibility of symposia or papers involving dairy economics and management.

13. *Dr. Hunziker memorial.* Voted that the consideration of the proposal for participation in the Dr. Hunziker Memorial be delayed until more specific plans are submitted and that action then be determined by mail vote of the Board.

14. *Sustaining membership.* Voted that a committee be appointed by the President to investigate the role of sustaining membership in other scientific societies and the probable impact of such members on the A.D.S.A. and report back at the next Annual meeting.

15. *Keeping Administrative Handbook up-to-date.* The Secretary must refer changes in the handbook to the Board before final action is taken.

16. *Nomination of officers; By-Laws amendment.* Voted that the by-laws regarding the nominating committee be approved as they appear on page 753 of the May, 1960, JOURNAL OF DAIRY SCIENCE.

17. *Length of service of committee members.* To broaden the participation of membership, it was voted to limit membership on board and section committees to six consecutive terms of one year, three consecutive terms of two years, or two consecutive terms of three years appointment, unless there is a compelling reason to extend it.

18. *Duties of committees and follow-through.* The President is to make clear to committee appointments that (a) rquests for interim meetings be made to the President and Secretary-Treasurer whenever it is deemed necessary; (b) that due dates for reports be emphasized when called for by Board action; (c) that annual reports to the Board contain not only a statement of accomplishments of the committee but recommendations for Board action if any be required.

19. *Enlargement of Education Committee.* Voted that the Education Committee be made a six-man committee.

20. *Approval of new Dairy Science clubs.* Voted that it should be the policy of the Board to extend a charter, when requested, to student branch clubs that might represent combined interests including dairy science where such clubs had provision in their constitution or by-laws for a member to represent the dairy science area.

Voted that a request for a student branch at Fresno State University be granted.

21. *New test or method reference committee.* Voted that the members and committees of the American Dairy Science Association be guided by the final action of A.O.A.C. with reference to the use of the Te Sa test or other tests within the jurisdiction of A.O.A.C. Voted, also, that American Dairy Science Association approval of any program, method, test, or procedure shall be contingent on favorable action by the membership at its Annual meeting upon recommendation by the Executive Board.

22. *Constitution and By-Laws.* Voted that the Executive Committee review the Constitution and By-Laws before acting upon any changes that may be necessary at the 1961 Annual meeting. Following any such action the revised Constitution and By-Laws to be printed in the JOURNAL, with reprints to be

made for distribution to new members or to others as may be required.

23. *Section chairman reports.* The chairmen and vice-chairmen of each section appeared before the Board and reported on the committee reports to be presented at their various business meetings. The action of the Board with reference to American Dairy Science Association approval of any program, method, test, or procedure was explained to the section chairmen. The plan of having the section chairmen and vice-chairmen attend designated meetings of the Board proved to be a good one and should be continued.

24. *Section Business Meetings.* Voted that the Program Committee consider the possibility of arranging the program so that there will be a lapse of one-half to one day between the business meeting of the section and the business meeting of the Association.

25. *Publication of Standard Methods for Milk Analysis.* Recommended that Dr. Luther A. Black be authorized to negotiate with the American Public Health Association and other technical societies in the arrangements for the future publications of the standard methods for the examination of milk and dairy products on a cooperative basis and that, before final arrangements are made, the proposal be submitted to the Board of Directors of the American Dairy Science Association.

26. *Awards.* Voted to ask the Policy Committee to study the limits of the areas of interest in the field of awards and the number and kinds of awards that would be within and without that area of interest.

27. *Program plans for 1962 Annual Meeting.* Voted that it is the feeling of the Board that at the 1962 meeting, special time on the program should be provided for appropriate recognition of the centennial of the founding of the land grant college system and the U.S. Department of Agriculture and that a special committee of five members consisting of the secretaries of the three sections for 1960, one member from the University of Maryland, and one member from the Department of Agriculture, be appointed to make advance plans and to work closely with the Program Committee of the Association concerning these plans.

28. *Selection of Secretary-Treasurer.* The Board voted to re-elect H. F. Judkins as Secretary-Treasurer of the American Dairy Science Association, with special thanks for his continued devotion and dedication to the work of the Association.

29. *Selection of Editor.* The Board voted to re-elect E. O. Herreid to the position of Editor-in-Chief of the JOURNAL OF DAIRY SCIENCE for 1961, with special thanks for the outstanding job he continues to do as Editor.

30. *Selection of Historian.* The Board voted that Dr. G. Malcolm Trout shall be the Historian.

31. *Selection of Journal Management Committee member.* The Board elected Harry Herman to this committee.

32. *Selection of Policy Committee.* The Board elected Ira A. Gould, Ohio, Chairman; George W. Werner, Wisconsin; and H. B. Henderson, Georgia, as new members.

33. *Selection of Program Committee Chairman for 1960-61.* Voted that George Trimberger be selected as the Program Committee Chairman for 1961 meeting.

34. *Representative to National Research Council.* Voted to ask W. E. Krauss to continue as National Research Council Representative for another year.

35. *Size of Student Affiliate Committee.* Voted that in event the Student Affiliate Committee desires to increase the size of its committee from six to nine members on a rotational basis, that the President be authorized to make such appointment.

36. *Awards Selection Committees.* Voted that in the future selection of members to serve on awards' committees, the element of academic or industrial retirement be disregarded and that the handbook be corrected accordingly.

37. *Awards to foreign members.* Voted that the President explore the attitude of donors of cash awards to members in foreign countries, should such members be selected for specific awards.

38. *Commendation for President Fay.* Voted that the Executive Board commend the retiring President, A. C. Fay, for inspiring leadership during the past year and wish him many years of happy association in the work of A.D.S.A. in the future.

39. *Appreciation to retiring Board members.* Voted that the Board express its appreciation for the service rendered by retiring members, H. A. Herman and K. L. Turk, and wish for them many more years of life and service in A.D.S.A.

EXECUTIVE BOARD ACTION ON COMMITTEE REPORTS

1. *Association Policy Commitee.* The Board accepted the report of the Policy Committee with the following recommendations:

(1) Business Organization of the Association. Voted to accept action on this problem as follows: (a) That we continue the present organization; and (b) that an Executive Committee, consisting of the President, Vice-President, and one of the members of the Board (to be selected by the President), with the Secretary-Treasurer and Editor serving in an advisory capacity, and such other persons as the President may find desirable to include, make a study

and submit recommendations for a specific plan of reorganization to be available for adoption when we can no longer continue the present setup. Due date for the preliminary report is the 1961 Annual meeting.

(2) Journal Management Committee clarification of responsibilities. Matter covered in Journal Management Committee report.

(3) Public Relaticns: Cooperation with the Dairy Industries Supplies Association. Voted that the committee on judging dairy products of the Manufacturing Section study and report to the Board on possible methods of improving the method of granting this award.

(4) Public Relations: The Philosophy of the A.D.S.A. Voted that the Resolutions Committee be composed of one representative from each section appointed on a three-year rotating basis, with the senior member serving as chairman. To establish the sequence of rotation in the beginning, the representatives to serve three, two, and one year, to be determined by chance. In appointing members to the committee, the President shall consult the new chairman from the section from which the appointment is to be made.

(5 and 6) Public Relations. (a) Public Relations Committee. In view of the fact that the Board voted to discontinue the Public Relations Committee at its 1958 meeting and agreed to substitute for it a Publicity Committee composed of the Secretary-Treasurer and the Editor and one other member of the Board, and in view of the fact that all members of the Association should play an important part in public relations, no recommendation is made for a change in our present procedure at this time. It is referred back to the Policy Committee for it to outline the need for a Public Relations Committee with a well-defined purpose and program. (b) Communication between members and executive officers. Voted to accept Policy Committee's report of recommendations 1, 2, 3, and 5, reading as follows:

1. That the section,[1] *Our Association,* be in the same place in the JOURNAL each month and preferably near the front of the JOURNAL, where it may attract reader attention. This section may carry statements by the President, other officers, the Secretary, the Editor, Chairman of committees and of members on matters of importance in the life of the Association. The Editor should assume responsibility in this matter.

2. Members should be invited through the JOURNAL to write to the Editor or to an officer expressing opinions on Association affairs, with assurance that letters will have proper attention. It was felt that each issue of the JOURNAL should carry an invitation for membership communication. Following are some forms of communication: a) Letters to the Secretary or Editor or officers will be answered or referred to the proper committee or individual officer; b) members may write directly to a committee; c) a communication may be directed to any officer of any section, to any director, or member of the Executive Board; d) letters to the Editor may be published or may be referred to the Secretary. Some letters may have to be answered by referring them to appropriate committees, which may be done by the Editor or the Secretary.

3. When committees make recommendations to the Executive Board which are not approved, the Secretary should notify the chairman of the committee the reasons for disapproval. Also, where the Board may modify and then approve the modified committee report, reason or reasons for such modification should be given to the chairman of the committee.

5. It is suggested that a plan be developed to edit and publish section committee reports.

Voted that in lieu of Recommendations 6 and 7 of the Policy Committee report the following recommendations are made: (1) That section committee reports be submitted in advance and reviewed by section chairmen and vice-chairmen; (2) that section chairmen and vice-chairmen be invited to meet with the Executive Board for one session to review: (a) the items of general interest, and (b) the resolutions of interest or those that might be controversial; and (3) that section chairmen be allotted five minutes each to review the important section matters at the Annual Business Session of the Association.

(7) Public Relations: International Relationships. Voted that the Executive Board designate the President to appoint a committee of not more than five to explore ways and means to achieve better international relations with dairy scientists over the world.

(8) Should a Teaching Award in Dairy Manufacturing and a Teaching Award in Dairy Production be offered each year? Voted that at this time the method of recognizing outstanding teachers in dairy production and manufacturing should not be changed. This question, however, should be reviewed frequently, considering the size of the Association, the number of members and their interests, and other pertinent factors, including financial support.

(9) Academic Freedom and the American Dairy Science Association. Voted to accept the recommendations, which are as follows: (1) Article II of the constitution of the A.D.S.A. subscribes to the principles of academic freedom, (2) the A.D.S.A. has no machinery to handle faculty complaints, (3) complaints of members involving principles of academic free-

[1] This section was designated for its present position in the JOURNAL when W. M. Roberts was Chairman of the Journal Management Committee and it has consistently appeared there.—Editor-in-Chief.

dom should be referred to the AAUP, (4) the Secretary should be directed to write a letter to Prof. E. Grant Moody, informing him personally of the action of the Board.

(10) Unity of the American Dairy Science Association. Voted that the Board approve the recommendation of the Policy Committee and that the Program Committee in cooperation with the host institution develop plans for holding an opening session at the Annual Meeting.

(11) Presidential Plaque. Voted that the Executive Board present an apropriate plaque at the Awards Ceremony to all future presidents when they leave that office, and that this plaque be given to all living past-presidents.

(12) Modernizing Constitution. Voted to accept the recommendations of the Policy Committee concerning two points: (1) A standing committee for keeping the Constitution and By-Laws modernized is not justified and (2) changes in the Constitution and By-Laws can be made when the need becomes apparent.

Voted that the Board request the Policy Committee to review the objectives of the Association in terms of future needs and developments.

Voted that the Executive Board give Dr. Walter Price a standing vote of thanks for a job well done for the past three years he has served as Chairman of the Policy Committee.

2. *Journal Management Commitee.* The Board approved the report of the Journal Management Committee with certain amendments, and recommends the publication in the JOURNAL OF DAIRY SCIENCE of that portion of the report dealing with actions taken during the year, after it has been revised and edited in JOURNAL form.

3. *Report of the Editor.* Accepted the Editor's report and voted that it be published in a condensed form in the JOURNAL.

4. *Annual Meeting Publicity.* Adopted the Annual Meeting Publicity Committee report.

5. *Publication Policy Committee.* This special committee is discharged with thanks for its efforts, and the Secretary is instructed to so advise the members of the committee.

6. *Secretary-Treasurer's Report.* Accepted the Secretary-Treasurer's report with a vote of thanks for a job well done.

7. *Budget.* Voted to amend the 1960 budget as indicated, making the total $93,883 instead of $94,223.

Voted to accept the proposed budget for 1961, amounting to $97,608.

8. *Auditing.* The Board approved the report of the Auditing Committee, which indicated a balance for 1959 of $3,221.56.

9. *Public Health Committee.* Voted that the report be accepted with commendations.

Inasmuch as the public health phase of dairying is of vital significance to the welfare of the human population and to the economy of the industry, this report merits special consideration. In the area of cattle diseases relating to human health the Board wishes to direct attention to the needs as set forth in several statements by the Committee:

First, to curb the apparent increase in tuberculosis reactors among cattle, special efforts should be given to accepted control practices, and research should be stimulated to improve positive means of detection of the disease.

Secondly, greater efforts should be placed on the elimination of brucellosis.

Thirdly, since mastitis persists as a major problem, concerted efforts of federal, state, and local agencies, in collaboration with all segments of the dairy industry, should be made to develop and maintain sound prevention programs.

In the area of antibiotic residues and chemical contaminants, primarily from pesticide usage, the Board wishes to commend the Committee for its analysis of the problems and to endorse the statement that emphasis be given to inspection, to testing, and to enforcement of regulations relating to milk and, that in the use of pesticides, labelled directions be followed closely. The need is recognized for an intensified educational program to inform the producers about proper measures to eliminate contamination and to allay the fears of the consuming public about the true status of milk as a source of health-impairing chemicals. To this end, the Board wishes to recommend that special reports be prepared either by the Public Health Committee or by some recognized authority in the field.

The effects of environmental radiation studies on milk are presented as information and not as a current problem. This phase of public health as it relates to the dairy industry has been covered adequately in recent reviews in the JOURNAL OF DAIRY SCIENCE.

The Board voted that this report be referred to the Secretary with instructions to resubmit it to the Public Healh Committee requesting preparation for publication of educational information on this subject.

10. *National Membership Committee.* The Board voted to accept this report with commendations for a job well done.

11. *Education Committee.* Voted to accept the report of this committee. The Board suggests that the Education Committee develop a strong and appealing program.

In order that the Education Committee may be able to obtain a more favorable situation for the presentation of their part of the program, it is voted that the Chairman of the incoming Education Committee be advised to prepare, at an early date, a specific program of an appealing nature that may be submitted to the Chairman of the General Program Committee prior to the Chicago meeting.

12. *Dairy Remembrance Committee.* The Board authorizes the President to discontinue the Dairy Remembrance Committee if after consultation with the Chairman it is deemed advisable.

13. *Report of Representative to the National Research Council.* Voted that this report be accepted, with special thanks to Dr. W. E. Krauss, and that the report be published in the JOURNAL OF DAIRY SCIENCE.

Special attention is directed to two items indicated by Dr. Krauss: First, that the A.D.S.A. give consideration to the impact of grants and fellowship programs on the quality of research and teaching. Secondly, that our Association give consideration to problems that might be placed before the National Research Council to receive the benefit of judgment from all scientific disciplines.

Voted that a consideration of the impact of the grants as set forth by Dr. Krauss in the National Research Council Report be referred to the Policy Committee for some study.

14. *Report of Historians of A.D.S.A.* Voted to accept this report with commendations to our Historian for his unflagging zeal.

Voted that the Journal Management Committee give consideration to the possibility of including, in a prominent part of our JOURNAL, biographies of pioneers in the dairy industry.

15. *3-A Standard Committee.* The Board considers the relationship between A.D.S.A. and the 3-A Standards Committee desirable. Voted: (a) that this committee be continued, (b) that its report be accepted, and (c) that the work of the 3-A Standards Committee be commended.

16. *Report of Ad Hoc Committee to draw up rules for distinguished service award to be offered by the Association.* Voted that the report on the procedures of the Distinguished Service Award be accepted with certain amendments in the proposed rules. A selection committee is to be appointed, looking forward to the granting of the first award at the 1961 meeting.

17. *Program Committee.* Voted to accept the Program Committee Report, with certain suggestions.

Report was commended, and it was suggested that Committee continue to meet at A.D.S.A. Annual Meeting to plan for coming year's program. Also, suggest that Program Committee continue to meet in November. It is evident that more detailed instructions for preparation of abstracts for the A.D.S.A. program are needed. Should be re-emphasized that papers submitted for the Annual Meeting should be confined to previously unreported research.

It is suggested that the Student Branch Section and its program should be the responsibility of the Student Affiliate Committee. A member of this Committee should work with the General Program Committee.

18. *Student Affiliate Committee.* Voted to accept the report of the Student Affiliate Committee except for Item 6. We feel that the constitution and by-laws submitted for the National Student Branch should be referred back to the Student Affiliate Committee, inasmuch as there seems to be a lack of unified opinion among those formulating this document. There is a lack of clarity of purpose and some ambiguity in the different sections.

Voted that the Chairman of the Student Affiliate Committee, accompanied by the student officer of the branch, be invited to attend a session of the Board meeting to discuss mutual problems.

19. *Report of Representative to A.A.A.S.* Voted to accept the report of A.A.A.S.

20. *Purina Fellowship.* Voted to approve the report and suggest that more dairy departments urge their students to apply for these fellowships.

21. *U. S. Livestock Sanitary Association.* Voted to approve this report, with a suggestion that the following papers which have appeared in the J.A.V.M.A., 136:4. February 15, 1960, be referred to by title and reference in the proceedings: *Field Control of Anaplasmosis,* L. O. Mott, E. E. Saulman, T. O. Roby, W. H. Martin, Washington, D.C. and Beltsville, Maryland; *Comparison of Serological Tests for Bovine Leptospirosis,* Leif Ringen, Pullman, Washington; and *Tuberculin Sensitivity in Wisconsin Cattle,* D. T. Berman and C. A. Tervola, Madison, Wisconsin.

22. *N.A.A.B. Report.* Voted to approve the report, and urge that strong cooperative working relationships continue.

23. *International Dairy Congress.* Voted to approve this report. Though unsuccessful, the Board commends the committee for its efforts to bring about a working relationship with the International Dairy Federation. In light of the difficulties involved, the work of the Committee may be considered to be finished.

24. *Censusing Procedures for Rangelands.* Voted to approve the plan as submitted for the censusing procedures for rangelands and that information of this action be submitted to Dr. J. T. Reid.

25. *Report of the Resolutions Committee.* The Resolutions Committee, after considering the various suggestions made by committees and sections of the American Dairy Science Association, plus those received from the heads of dairy departments of colleges and universities, submits the following resolutions which have been approved by the Board of Directors:

WHEREAS, the members of the American Dairy Science Association who participated in the 55th annual meeting found the arrangements and accommodations in Logan most satisfactory and enjoyable, therefore it is

1. RESOLVED, that the American Dairy Science Association express its sincere appreciation to the staff of Utah State University, particularly the members of the Dairy Industry Department and their families, for their efforts and contributions toward making this meeting successful and for the hospitality which they showed us. We also wish to thank the various government agencies and industry firms and other groups who made special arrangements on the occasion of our Association's visit to Utah.

WHEREAS, scientific workers and teachers recognize the value of moral and financial support from industry, therefore it is

2. RESOLVED, that the American Dairy Science Association express its appreciation to the Borden Company, the American Feed Manufacturers' Association, the De Laval Separator Company, the Milk Industry Foundation, the National Dairy Products Corporation, and the Paul Lewis Laboratories for the awards given annually to members of this Association in recognition of outstanding work in research, teaching, and extension.

WHEREAS, for many years Dr. G. Malcolm Trout served as chairman of the Committee for the Students' Dairy Products Judging Contest and in this position by his skill and devotion contributed to the high standards and significance of this event, therefore it is

3. RESOLVED, that the American Dairy Science Association express its appreciation to Dr. Trout for his untiring efforts toward this cause, for the enthusiasm with which he has advanced the art of judging dairy products, and for his excellent liaison with the Dairy Industries Supply Association.

WHEREAS, adequate and well-defined standards for milk handling and processing equipment have contributed much to the wholesomeness, safety, and high quality of milk and milk products, therefore it is

4. RESOLVED, that the American Dairy Science Association continue its support of and participation in the work of the 3-A Standards Committee.

WHEREAS, the use of milk and milk products in milk-deficient areas of the world is important to the health and well-being of man and promises to provide outlets for milk products manufactured in the U.S.A., therefore it is

5. RESOLVED, that the American Dairy Science Association commend the many firms and individuals, and the USDA which, through Dairy Society International, has pioneered the attempts to introduce the use of milk products among the undernourished peoples in the world.

WHEREAS, the eradication of brucellosis is of vital importance to the dairy industry and to the health and welfare of the people of the United States, and

WHEREAS, the fight against this disease is handicapped by limitations of federal funds appropriated for the eradication program, therefore it is

6. RESOLVED, that the American Dairy Science Association urge the U.S. Congress to appropriate adequate funds to advance the program of eradicating brucellosis, and be it further

7. RESOLVED, that a copy of this resolution be mailed to the Secretary of Agriculture and to the members of the appropriate committees of the Senate and House of Representatives of the United States.

WHEREAS, mastitis is a major economic loss to dairy farmers and the dairy industry, therefore it is

8. RESOLVED, that the American Dairy Science Association urge federal, state, and local authorities and the dairy industry to review their activities related to the elimination of mastitis, and to undertake and expand positive programs of research and education for the prevention and control of mastitis in dairy cattle and to encourage the development of means of avoiding the presence of antibiotic residues in milk.

WHEREAS, the use of chemicals for the control of insects on milk cows and farm crops and for eradication of weeds is necessary to the economic production of milk, and

WHEREAS, by improper application, residues of these chemicals may appear as adulterants in milk and milk products, and

WHEREAS, various federal agencies are waging a vigorous campaign against adulteration of all foods, therefore it is

9. RESOLVED, that the American Dairy Science Association commend the federal, state, and local authorities, the extension service, and the dairy industry for carrying out vigorous programs of assistance and education in the proper and safe methods of using these chemicals by milk producers and handlers. However, representatives of the above agencies and institutions are urged to strengthen their programs of research and education pertaining to this problem. Attention should be directed particularly to the hazards from pesticide residues in feeds produced in areas remote from the place of use and not under direct control of the milk producers.

WHEREAS, continued improvement in the general quality of dairy products is in the best interest of the dairy industry, therefore it is

10. RESOLVED, that the American Dairy Science Association support in principle the establishment of minimum quality standards and specifications for the production and processing of milk for manufacturing purposes.

WHEREAS, certain animal feed additives improve the efficiency of production, quality of the end product or total yield of the product,

or are valuable in maintaining the health of animals, and

WHEREAS, the Delaney clause in the 1958 food additives amendment to the Food, Drug, and Cosmetic Act prohibits the use of any additive to human food if the specific additive is known to cause cancer in any species by any dose, denying the Food and Drug Administration the use of scientific judgment in individual cases, thereby resulting in unreasonable prohibitions against potentially valuable additives, therefore it is

11. RESOLVED, that the American Dairy Science Association urge the revision of this amendment by Congress to empower the Food and Drug Administration to accept or reject an application after evaluating the scientific evidence and opinions developed by its own and outside experts concerning the potential danger of such additives to consumers and for the use of specific feed and food additives.

BE IT FURTHER RESOLVED, that the Secretary of this Association be instructed to forward a copy of this resolution to the Secretary of Health, Education, and Welfare, the Secretary of Agriculture, and the members of the appropriate committees in the Senate and House of Representatives of the United States.

WHEREAS, efficiency in milk production is important to the individual milk producer, therefore it is

12. RESOLVED, that the American Dairy Science Association again strongly urge that increased support be given to the Dairy Husbandry Research Branch of the USDA, to advance the production-testing and sire-proving programs now under way in the United States.

WHEREAS, vertebrates are vital organisms used in research relating to livestock production and human welfare, and

WHEREAS, their use has advanced our knowledge in all fields of biological science and without undue inhumane methods, and

WHEREAS, their future use in research is of increasing importance, therefore it is

13. RESOLVED, that the American Dairy Science Association strongly oppose the passage of Senate Bill 3570, inasmuch as this bill imposes unnecessary obstacles in the execution of important and needed research for livestock production and human welfare, and be it further

14. RESOLVED, that a copy of this resolution be dispatched to the Majority Leader in the U.S. Senate, the Speaker of the House of Representatives, and to the Secretary of Agriculture.

V. H. Nielson, Iowa, Chairman
G. C. Anderson, Idaho
E. E. Bartley, Kansas
L. C. Thomson, Wisconsin
D. H. Williams, Washington, D.C., Dairy Industries Supply Association

REPORT OF THE EXTENSION SECTION

The business meeting was held at 10:30 A.M. on June 20, 1960, with Chairman C. W. Nibler presiding.

Officers elected for 1960-61 include: Chairman, Marvin E. Senger (North Carolina); Vice-Chairman, W. R. Van Sant (Arizona); and Secretary, C. D. McGrew (Ohio).

The following committee reports were presented and accepted:

DAIRY RECORDS

The Dairy Records Committee in cooperation with the Program Committee developed the program for the first morning of the Annual Conference of the Extension Section. The Executive Committee of the American Dairy Science Association was asked by the Dairy Records Committee to name a committee which would evaluate all new equipment used by the dairy industry. This committee would be permanent in nature and would designate institutions for study of the new devices. New tests and new devices have become too numerous for the Dairy Records Committee to handle.

A questionnaire on the use of preservatives in DHIA milk samples was sent to all states. The preservatives used were sodium dichromate, potassium dichromate, mercuric chloride, and formalin.

Extension Section officers in action. Chairman, C. W. Nibler and Secretary, W. R. Van Sant.

The committee accepted DHIA as being a part of the National Cooperative Dairy Herd Improvement Plans. The committee recommended that the 1961 Record Committee appoint a committee to develop transfer forms and present them at the annual meeting of processing centers.

The vocational agriculture testing program was recommended to be reported as owner-sampler records.

The committee initiated tests at Kentucky and two independent laboratories to run check tests to evaluate the effect of preservatives on the comparative accuracy of the Babcock and TeSa methods in the determination of butterfat content in composite preserved milk samples. The Kansas State University Department of Statistics, Statistical Laboratory, made the statistical analysis. The results showed that the two tests gave equally reliable results. The Dairy Records Committee approved the use of TeSa test for use in dairy production record-keeping plans with fresh milk or milk preserved with mercuric chloride, sodium diehromate, potassium dichromate, and formalin at recommended concentrations.

A complete revision of the Standard DHIA rules was made, based upon the recommendations of State Extension Dairymen in keeping with the developments in the testing program.

4-H CLUB

The 4-H Development Committee is working on ownership requirements of cattle for showing at fairs.

The 4-H Committee worked with Extension Program Planning Committee and gave recommendations for speakers on the subject of Type and Its Importance in the 4-H Dairy Program. Two papers were presented: (1) Importance of Type in Dairy Cattle Breeding and Management, (2) The Value of Junior Shows in Relation to the Growth and Development of the Guernsey Breed.

TEACHING METHODS

Eight states presented exhibits on effective ways and methods of extension teaching. Two papers were presented: (1) Salesmanship Schools for DHIA Supervisors, (2) Role of the Dairy Specialist in a Changing Agriculture. A panel discussion was presented on Preparing and Packaging Visual Aids for County Workers.

RESOLUTIONS

The Extension Resolutions Committee presented the resolutions developed by the overall committees and they were accepted as presented. In addition, the Extension Section requested a resolution be added requesting that the JOURNAL continue to publish the Interpretive Summaries.

A total of sixteen papers were presented in the general areas of production testing, feed evaluation, sire proof evaluation, mastitis, antibiotics and pesticides. Abstracts of papers have been published and copies have been distributed to all extension dairymen.

REPORT OF THE MANUFACTURING SECTION

Two business meetings of the section were held on Monday, June 20, 1960, Chairman Stuart Patton presiding. The report of the Nominations Committee was presented by J. C. Flake, Acting Chairman. The following officers were elected for the coming year: Burdet Heinemann, Chairman; Marvin L. Speck, Vice-Chairman; D. M. Graham, Secretary.

In the report of the Resolutions Committee, presented by W. M. Roberts, Chairman, special thanks were given to Dr. G. M. Trout for his leadership in promoting the growth and influence of Collegiate Students' International Contest in Judging Dairy Products, and for his encouragement to the many contestants of this outstanding Contest. Also, thanks were extended to Dr. P. A. Downs for his invaluable service to the aforementioned Collegiate Contest, and a record 30 consecutive years of coaching and entering judging teams in this Contest. Special commendation was given to Dr. Trout's 21 years as Chairman of the Committee of Judging Dairy Priducts, A.D.S.A., and to Dr. Downs' membership of 32 years on this Committee. The Section enthusiastically approved the report of this committee.

The activities of the Committee on Judging Dairy Products, A.D.S.A., were reported by E. L. Thomas, Chairman. Possible changes were discussed regarding sample preparation and the use of samples having wider ranges of score in the Contest. Note was also made of the declination of scholarships by Contest winners and the need for increasing the stipend to winners. The report was approved and the Committee continued.

The report of the new Committee to Develop a Score Card for Sterile and Concentrated Milks was presented by J. C. Flake, Chairman. Work was begun on a score card for use on concentrated milks for beverage purposes. The report was approved and the Committee continued.

Dr. C. A. Zittle, Chairman, reported for the Committee on Nomenclature and Methodology of Milk Proteins. The increasing literature on α-casein complex and related subjects will be surveyed by the Committee, and it will keep the Association advised regarding nomenclature recommendations. The report was approved and the Committee continued.

The report of the Butter Committee was presented by Claude Harper, Jr., Chairman. This Committee has agreed in principle on a program for a change of the present legal definition of butter to a standards of identity for butter under the Food and Drug Administration. This report was approved and the Committee continued an additional year, in order to obtain a wider view of this significant change.

Manufacturing Section officers in action. Vice-Chairman, Burdet Heinemann, Secretary, M. L. Speck, and Chairman, Stuart Patton.

Owing to the increased number of papers being presented at the annual meeting, and the consequent need for more concurrent sessions, it was moved that a vote of confidence be extended to the Chairman to appoint more assistance in leading the subsections. The motion carried.

Possible topics for symposia next year were presented.

REPORT OF THE PRODUCTION SECTION

The Production Section Business Meeting was held June 20 with Chairman George Trimberger presiding. Minutes of the 1959 meeting were read and approved. Officers elected for 1960-61 were: Chairman, S. D. Musgrave (Oklahoma); Vice-Chairman, J. C. Thompson (Missouri); and Secretary, L. H. Schultz, (Wisconsin).

The Dairy Cattle Judging Committee report was adopted as presented. It included the results of a questionnaire that had been sent to the coaches of the schools that normally compete in the national contest in Waterloo. The following recommendations were prepared by the committee from that questionnaire:

1. Bull classes continue to be a part of the national dairy cattle judging contest.
2. The following statement to be added to the eligibility requirements for team participation in the national intercollegiate dairy cattle judging contest: New applications for participation in the national contest must be submitted to the Dairy Cattle Judging Committee of the American Dairy Science Association by February 1 of the current year's contest.
3. The Kildee Scholarship Committee of the Dairy Shrine Club be encouraged, through the Dairy Cattle Judging Committee of the American Dairy Science Association, to expand the number of qualifications as a basis for awarding the Kildee Scholarship.

The report of the Resolutions Committee, expressing appreciation to the staff members of the Dairy Department of Utah State University, to the Section officers, and to the various committee members for their respective contributions to the success of the program, was adopted.

Chairman Trimberger reported that a total of 128 papers were included in the program, of which 11 were by graduate students competing for the $100 award. The authors were from 35 states and from England, Canada, and Colombia, South America. Two invitational papers and participation in two symposia were among the high lights of the 1960 meeting. Chairman Trimberger also expressed appreciation for the fine cooperation of the authors in getting material to him in record time this year.

A joint meeting of Production and Extension Section was held at 5 P.M. on June 20. Reports of the Dairy Cattle Breeding Committee, the Type Committee, and the Dairy Cattle Health Committee were all adopted as read.

The major factors included in the Dairy Cattle Breeding Committee report were:

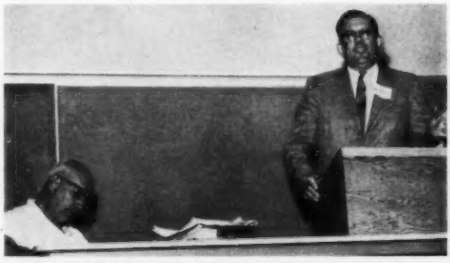
(1) Ideas on cattle abnormalities
(2) Greater emphasis on protein, lactose, mineral, and vitamin values of milk
(3) Importance of keeping sire proofs up to date
(4) Need for earlier information of breeding values of young sires.

The Type Committee report included:

(1) Dropping of bull classification, and lowering as well as raising females on successive inspections
(2) The importance of additional research concerning the correlation between longevity and type
(3) Limiting ages of bulls in show ring classes to not more than 2-year-olds.

The Dairy Cattle Health Committee presented a voluminous report on a number of subjects too lengthy to summarize here. It was a very important report and should be reviewed in detail rather than abstracted.

The new Chairman, S. D. Musgrave, announced that committee members would be notified by mail at a later date.



Production Section officers in action. Secretary J. C. Thompson and Chairman G. W. Trimberger.

REGISTRATION AT ANNUAL MEETING

Men	
Members	567
Affiliates	135
Nonmembers	113
	815
Women	364
Children	454
Total	1,633
Number of men registering from universities	504
Other male registrants	311

Eighty-eight per cent of those attending preregistered.

The following foreign countries were represented: Australia, Canada, England, Indonesia, Iran, Ireland, Israel, Italy, and Puerto Rico. Hawaii, the 50th state, was also represented.

NECROLOGY REPORT

Reuben W. Brown, Canada
M. L. T. Ellis, Hawaii
C. E. Gordon, California
C. C. Hayden, Ohio
I. L. Hathaway, Nebraska
O. H. Hunziker, Illinois
Nandor Porges, Pennsylvania
L. S. Trunnell, Iowa

BOOK REVIEW

DAIRY INDUSTRY PLANT TRAINING MANUAL. The American Dairy Science Association. 127 pp. 1959.

This manual should be welcomed by everyone responsible for the training and development of personnel for the Dairy Industry. It is designed to systematically guide a promising employee through a period of training in the various departments of a dairy company, and to provide him with suggestions and procedures so that he may continue to improve at the supervisory level.

Specific training programs are outlined for the main segments of the dairy processing and manufacturing industries, including milk, ice cream, condensed and dry milk, evaporated milk, butter, and cheese operations. Each training program is divided into sections representing the major departments of an organization, and specific assignments are given for the trainee to follow in each department.

A training program involves the following operations: (1) the assignment of the trainee to a supervisor who will serve as the principal counsellor throughout the training period; (2) the direct participation by the trainee in the planned program by following the assignments as outlined; (3) the adoption of a definite time schedule for the work and training assignments; (4) the use of the question-and-answer approach to guide the trainee in the departmental phases of the program; and (5) the application of a periodic report and evaluation plan.

It is emphasized that the material presented is intended to be highly flexible from the standpoint of adaptability to (1) men with different educational backgrounds, (2) plants of different sizes, and (3) programs of different lengths.

This manual is the result of approximately 3 yr. of extensive planning and work by The Personnel Training Manual Preparation Committee of the American Dairy Science Association, composed of eminently qualified leaders active in the educational and operational phases of the dairy industry. It is the expressed conviction of the authors that effective use of the manual by management will reap benefits in the form of greater profits through better qualified and more contented personnel—the most valuable asset of the Dairy Industry.

In addition to serving as a training tool for industry, this manual will serve as a valuable supplement in college courses in plant management and may be used as a guide in the preparation of material for summer dairy plant experience programs offered by many schools as part of the dairy industry curriculum.

E. L. THOMAS
University of Minnesota
St. Paul

ERRATA

A. C. Linnerud and J. D. Donker. J. Dairy Sci., 43: 879. 1960. p. 70, third paragraph, line 7, should be (1) + .39; (2) + .17; and (3) + .47, respectively.

D. H. Bullock and W. C. Winder. J. Dairy Sci., 43: 301. 1960. Table 4, page 310, last column heading should be 6° C.

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Bacto-Littman Oxgall Agar
Bacto-Mycological Agar
Bacto-Mycological Broth
Bacto-Corn Meal Agar
Bacto-Corn Meal Agar with Dextrose
Bacto-Bean Pod Agar
Bacto-Prune Agar
Bacto-Lima Bean Agar

CONTROL

. . . for sanitary and sterility procedures as well as for general use in mycological procedures:

Bacto-Sabouraud Maltose Broth
Bacto-Sabouraud Liquid Medium
Bacto-Malt Extract
Bacto-Malt Agar
Bacto-Neurospora Culture Agar
Bacto-Potato Dextrose Agar
Bacto-Mildew Test Medium
Bacto-W.L. Nutrient Medium
Bacto-W.L. Differential Medium

CLASSIFICATION

. . . and nutritional studies of fungi:

Bacto-Yeast Morphology Agar
Bacto-Yeast Carbon Base
Bacto-Yeast Nitrogen Base
Bacto-Czapek Dox Broth
Bacto-Czapek Solution Agar
Bacto-Vitamin Free Yeast Base

THE DIFCO MANUAL, *NINTH EDITION*,
including descriptions of these media and their use,
is available on request.

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